Fiber-optic catheter for cellular resolution imaging

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Fiber-optic catheter for cellular resolution imaging

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in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

2017

Statement of Originality

I hereby certify that the intellectual content of this thesis is the product of my original research work and has not been submitted for a higher degree to any other University or Institution.

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Date

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Dongyao Cui

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Dongyao Cui, August, 2017

Abstract

Patients with cystic fibrosis, primary ciliary dyskinesia and chronic obstructive lung disease suffer from airway narrowing and lung infection, leading to morbidity and mortality. Mucociliary clearance (MCC) is a critical self-defense mechanism that prevents respiratory system infection. Impairment of MCC is associated with alterations in the phenotype of epithelial ciliated, mucous, basal cells, etc. However, further understanding of disease pathogenesis and corresponding development for disease diagnosis therapeutics are hindered by lack of an imaging tool for *in vivo* visualization of these altered phenotype of ciliary function and MCC in real time. Currently available imaging tools cannot provide comprehensive parameters of interest, periciliary liquid (PCL), airway surface liquid (ASL) depth, ciliary beat frequency (CBF) and mucociliary clearance transport (MCT) simultaneously.

μ OCT has been known as the highest resolution OCT technique available to date and validated as a powerful tool to evaluation MCC and ciliary function. The unprecedented resolution enables quantitative assessment of all parameters of interests at the same time within one imaging session.

Several major developments were included in this dissertation to translate μ OCT towards clinical applications and advance μ OCT to higher resolution and better image quality. Four main topics were covered:

1. Visualization of cilia regeneration after injury.
2. Endoscopic μ OCT fiber catheter design and prototype toward *in vivo* clinical applications.
3. Dual spectrometer for *in vivo* imaging of blood flow.
4. Multifiber μ OCT catheter for speckle reduction.

We first studied cilia *in vivo* regeneration with μ OCT for a better understanding of the recovery process after certain diseases/accidents. We visualized adult mice tracheas up to 52 days after sulfur dioxide injury and quantified epithelial thickness, cilia coverage, length, beat frequency, coordination, and cilia-generated flow. We observed that regeneration of ciliated cells and cilia completed in 6 days but cilia gradually aligned and functioned in a coordinated way both on individual level and as a group for liquid transport. The later process takes longer. Notably, an overshoot in CBF was observed at the early stage when cilia just fully regenerated. It could be a sign of lag in cilia coordination at the early stage of regeneration. This study was described in Chapter 2.

As the previous study was also limited to excised tissues, Chapter 3 presents a novel high-resolution endoscopic μ OCT catheter, pushing the clinical feasibility of μ OCT a big step forward. The μ OCT catheter herein was designed and fabricated to perform longitudinal scanning *in vivo* in human airways. The outer diameter of 2.4 mm and flexibility allowed it to be inserted into the instrument channel of a standard bronchoscope. Under the real-time video guidance of the bronchoscope, the endoscopic fiber-optics catheter could reach the regions of interest up to secondary bronchi and conduct high resolution, real time imaging of the functional microanatomy of the airway epithelium, including ciliary beat frequency and mucociliary transport rates, etc. We demonstrated the feasibility of the catheter using mouse trachea *ex vivo* and swine trachea *in vivo*.

The next two chapters are dedicated to improve spatial resolution and image quality of μ OCT based on benchtop systems.

Chapter 4 presents a dual spectrometer μ OCT system for extended spectrum bandwidth detection in order to achieve 1- μ m axial resolution. The dual spectrometer design makes it possible to improve axial resolution using NIR radiation and gain improved penetration by use of longer center wavelength. The dual spectrometer utilized two line scan cameras of distinct detection wavelength ranges. The two spectral ranges were combined into a supercontinuum with extended full-width-half-maximum of 345 nm and thus the axial resolution in aqueous environment was 0.93 μ m ($n = 1.37$). *In vivo* experiment showed blood flow in zebrafish larvae tail vein. Not only individual red blood cell was observed but also endothelial cells lining along the luminal surface of the blood vessel wall.

Although at 1 μm resolution μOCT has enough resolving power to identify sub-cellular of biological tissue, some important microstructures was masked behind speckles. With the objective to improve imaging quality at high resolution, a multifiber μOCT catheter was designed to reduce speckle, as described in Chapter 5. The key element in this design was a multi-facet fiber array which actively delivered three light beams onto imaging sample and picked up back-scattered signal at different angles. Simultaneous multichannel spectrometer was employed to detect signals from different channels. Speckle reduction was realized by compounding signals from all channels. Rat esophagus imaging using this setup demonstrated an improved signal-to-noise ratio, contrast-to-noise ration and equivalent number of looks. The epithelium was better delineated from the overlying mucus and lamina propria beneath the basement membrane.

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Abbreviations

AMI	Acute Myocardial Infarction
ASL	Airway Surface Liquid
CAD	Coronary Artery Disease
CBF	Ciliary Beat Frequency
CF	Cystic Fibrosis
CLE	Confocal Laser Endomicroscopy
CNR	Contrast-to-Noise Ratio
COPD	Chronic Obstructive Pulmonary Disease
DOF	Depth of Focus
ENL	Equivalent number of looks
EP	Epithilium
EtO	Ethylene Oxide
FWHM	Full Width Half Maximum
GI	Gastrointestinal
HBE	Human Bronchial Epithelial
ID	Inner Diameter
MCC	Mucociliary Clearance
MCT	Mucociliary Clearance Transport
MFOCT	Multi Fiber Optical Coherence Tomography
NIR	Near Infra Red
OCT	Optical Coherence Tomography
OD	Outer Diameter
μOCT	Micro-Optical Coherence Tomography

PCD	Primary Ciliary Dysinesia
PCL	Periciliary liquid
PEEK	Polyetheretherketone
PIT	Pathological Intimal Thickening
PSF	Point Spread Function
SDOCT	Spectral Domain Optical Coherence Tomography
SLD	Superluminescent Diodes
SNR	Signal-to-Noise Ratio
TCFA	Thin-Cap Fibroatheroa
ThCFA	Thick-Cap Fibroatheroma
WLE	White Light Endoscopy

Chapter 1

Introduction

1.1 Disease Imaging

1.1.1 Mucociliary clearance

Mucociliary clearance (MCC) is a critical self-defense mechanism of human respiratory system to filter inhaled foreign substances. Around 20% of epithelia lining along human lower respiratory tracts are goblet cells that produce mucus to trap foreign substances and particles. The other 80% of the epithelia are ciliated cells, which are covered by motile tiny hair-like structures called cilia to propel mucus and remove the trapped wastes out of human body. Cilia are surrounded by an aqueous environment as thick as the length of cilia, which is called periciliary liquid (PCL), to ensure effective ciliary motion. Mucus is transported by cilia in the direction from respiratory tracts distal end to proximal end. The thickness of the mucus layer depends significantly on the health condition of the respiratory system and varies from $2\mu\text{m}$ to $10\mu\text{m}$ for healthy airways [1]. The total thickness of the PCL and the mucus layer, referring as the airway surface liquid (ASL) depth, is one critical parameter to describe the respiratory health condition. Another metric is ciliary beat frequency (CBF), an indicator of cilia vitality in transporting mucus. The CBF is from 4 Hz to 12 Hz in healthy human [2] and is generally observed to be lower in patients with respiratory system diseases.

Many diseases are associated with failure in MCC, such as cystic fibrosis (CF), primary ciliary dyskinesia (PCD), and chronic obstructive pulmonary disease (COPD),

leading to morbidity and mortality. For example, CF is an autosomal recessive genetic disease, affecting around 30,000 patients across the U.S. It is often diagnosed at the first few years of life. But even with the most advanced therapies, the average life expectancy of CF patients is significantly reduced to 30 or 40 years. The patients also suffer from severe airway blockage and lung infections due to impaired MCC at their last times. While therapies exist, the pathogenesis and the mechanism of CF remain insufficiently understood, partially due to the lack of an imaging technology for studying MCC non-invasively in real time. The only technique to provide four critical metrics of MCC (CBF, PCL, ASL, and MCT) at the same time is μ OCT. However this technique is limited to excised tissue or cells. The ability to monitor MCC *in vivo*, in real time and over time would advance not only diagnosis of the diseases but also the development of corresponding novel therapeutics.

1.1.2 Coronary artery disease

Coronary artery disease (CAD), and its clinical manifestation, acute myocardial infarction (AMI), is the leading cause of death, claiming nearly 7.3 million lives worldwide annually [3]. In United States alone, the disease costs approximately USD154B in 2008 and will triple by 2030 if our ability to diagnose and manage the disease stays where we are right now [4].

Atherosclerosis is a disease of the arterial vessel characterized by both lipid uptake and the inflammatory response in the vessel wall. Acute Myocardial Infarction (AMI, = Heart attack) are caused by the rupture or erosion of atherosclerosis plaques present in the arterial vessel supplying the heart. The rupture of a vulnerable plaque causes the bloodstream to be exposed to procoagulant factors, forming thrombus [5]. The thrombus can impede blood flow to downstream myocardium, triggering an acute coronary event.

Currently we are limited in clinically viable methods to understand and predict which plaques will give rise to coronary thrombosis in any given patient [5].

Atherosclerosis lesions are generally classified based on their morphology as pathological intimal thickening (PIT), thin-cap fibroatheroma (TCFA), thick-cap fibroatheroma (ThCFA), fibrotic plaque, or fibrocalcific plaque. Importantly only

FIGURE 1.1: Photomicrographs of plaque rupture and thin-Cap fibroatheroma. Plaque rupture (**A and B**) and thin-cap fibroatheroma (**C and D**). Boxed areas in A and C show the thin fibrous cap, which is infiltrated by macrophages; B is rupture site, and D shows intact fibrous cap. [6]

thin-cap TCFA plaques are thought to be vulnerable to rupture and induce AMI. Morphological features important for the identification of a vulnerable “TCFA” are: 1) a lipid-rich necrotic core with 2) a very thin fibrous cap ($<65\ \mu\text{m}$ thick) and 3) presence of inflammation and macrophages infiltration in the cap [6, 7] (Figure 1.1). Identification of vulnerable lesions has become the “holy grail” of interventional cardiology with the idea that, if vulnerable site can be identified before rupture, we could then treat them by interventional means or even medically to prevent clinical events.

However attempts to identify plaques likely to rupture imminently using conventional imaging methods have mostly failed, partly because of their lack of resolution and specificity to detect cellular and extracellular structure like microphages, cholesterol crystals, and collagen fibers associated with TCFA [6]. For example, it is very difficult for the existing technologies to identify and quantify cellular content of the plaque cap such as macrophages, cholesterol crystals, smooth muscle cells, collagen and elastin fibers et al, which determines the vulnerability of the plaque. An Investigational tool, which can provide sub-cellular information, will greatly improve the detection of vulnerable plaques and therefore saves lives before

AMI events.

1.1.3 Gastrointestinal (GI) cancer

Esophageal cancer, gastric cancer and colorectal cancer are among the mostly commonly diagnosed cancers worldwide, claiming nearly 1.8 million lives and more than 2.5 million new cases per year worldwide [8]. Some cancers are characterized by tissue morphological changes. For example, Barrett's esophagus (BE) is a precursor lesion for esophageal adenocarcinoma. One of the most important characteristics of Barrett's esophagus is the replacement of the normal stratified squamous epithelium lining of the esophagus by simple columnar epithelium with goblet cells [9]. Besides, colorectal cancer is generally accepted as a progression from hyperplasia (benign adenoma) [10], and thus removing adenomatous polyps can likely reduce the risk of developing malignant tumors. Therefore, precise assessment of these morphology features as early as possible before they progress to an advanced stage is very important. Early diagnosis of GI cancers help physicians to intervene in advance, slowing or even stopping the course of disease development, improving prognosis, and decreasing mortality. Early GI cancer detection requires high resolution endoscopic imaging tools. For screening and surveillance endoscopy, biopsy materials are obtained with the visual guidance of standard white light endoscopy (WLE) and histology analysis of these biopsy materials remains the gold standard for a definitive diagnosis of lesions. However, as the early stage lesions are often confined within the lower half of the epithelium and may not be visible under WLE, detection and diagnosis of early neoplastic changes in GI tracts is extremely difficult with current standard WLE and random biopsy technologies. An endoscopic tool for obtaining histology-grade images from large epithelial surfaces within patients could enable screening and surveillance of intraepithelial tumors invisible to WLE, reduce the number of biopsies, and thereby, reduce the risk of complications and the cost.

1.2 State-of-the-art imaging techniques

Studies of the pathogenesis and diagnosis of above mentioned diseases depend on the development of advanced imaging technology to visualize biological structural lesions or tissue dynamic malfunctions at high resolution.

1.2.1 MCC imaging techniques

Fluorescence confocal microscopy has previously been used to study PCL and ASL in cell culture [11]. It can only provide static images due to point-by-point sampling of such technique. In addition, fluorescence dye should be introduced during imaging for contrast enhancement, which also introduces unclear effects of the fluorescence dye on tissue sample.

Electron microscopy is another imaging technique used to study PCL and ASL [12, 13]. It remains as the gold standard for PCD diagnosis. However electron microscopy is also limited to provide static images of cell culture or excised tissue. Further more, it is prone to tissue processing artifacts.

A comprehensive understanding of ciliary dynamic function is unavailable for these two imaging techniques.

High-speed video microscopy allows for dynamic information such as ciliary beat pattern and coordination. It also can be used to quantitatively assess the CBF [14, 15]. High-speed video microscopy is the current gold standard to evaluate dynamic ciliary function. The limitation is that this technique is not suitable to study MCC *in vivo* because optical depth sectioning is not allowed.

In a nutshell, the above mentioned techniques to study MCC are all limited to investigating excised tissue or cells, and typically provide only a single parameter of interest instead of a comprehensive picture of the interaction between mucus properties and ciliary function.

1.2.2 Confocal laser endomicroscopy (CLE)

CLE is one technology that is capable of providing images that contain cellular and subcellular features of the esophageal [16–18], gastric [17, 19], and colorectal epithelium [17, 20, 21]. In previous reports, CLE has been shown to distinguish squamous mucosa from gastric pits [22], Barrett’s esophagus with specialized intestinal metaplasia (SIM) from normal gastric or squamous epithelium [23], and neoplasia associated with BE from SIM [18]. Although CLE shows promise, its field size is usually limited to less than 500 μm [16–21, 23–25]. Thus, confocal endoscopic imaging may be subject to sampling errors similar to those of endoscopic biopsy [26]. Importantly, fluorescein-based CLE does not display nuclei, because of the pharmacokinetic properties of fluorescein. Nuclei are of particular importance for identifying types of tumor and judging low-grade and high-grade dysplasia [16, 17, 19, 27]. More importantly, the imaging plane depth of CLE is currently limited to 250 μm . The submucosal layer is inaccessible in many cases. Submucosal malignant invasion defines invasive cancer and therefore cancer grading and staging [17, 27], and as such, CLE is currently not well suited to diagnose invasive lesions. Lastly, comparison with standard histopathologic analysis is limited because the fibered confocal microscope produces images of horizontal planes, parallel to the surface of the GI tract, while vertical sections are displayed in routine histopathologic analysis.

1.2.3 Histopathology

Histopathologic analysis of biopsy tissues is the clinical gold standard for accurate diagnosis of these diseases. In histopathology, examination of tissue samples at cellular and subcellular resolution is often needed to make an accurate diagnosis. However, endoscopic biopsy is costly and associated with complications, placing substantial burden on the patients and the healthcare system. For example, it is very dangerous to conduct biopsy in Barrett’s esophagus and ulcerative colitis patients where the lesions have a patchy distribution throughout the entire organ. The situation is even worse, if possible, to conduct biopsy of coronary arteries. In addition, as histology requires workup and the evaluation is time-consuming, which may limit the ability of the endoscopist to immediately determine the necessity for intervention during ongoing endoscopy, possibly resulting in the need for

repeated endoscopies. Furthermore, biopsy forceps only sample a small fraction of the tissue under investigation, so that sampling errors leading to missed diagnosis are common. Redundant biopsies have to be taken for many lesions that are subsequently determined to be not malignant. Thus, pathologists must bear a heavy burden of the work, and patients incur increased costs and associated risks (such as bleeding). Moreover, because of local fibrosis after biopsy, endoscopic resection is difficult [6, 28].

An imaging tool for obtaining histology-grade images non-invasively could enable screening and surveillance of gastro-intestinal cancers and coronary artery diseases, reduce the number of biopsies, and thereby, reduce the risk of complications and the cost.

1.2.4 Non-invasive angiography

Non-invasive angiography techniques provide spatial resolution in the order of hundreds of microns, which is 2-3 orders of magnitude lower than that of histology. Catheter-based angiography tools have much improved spatial resolution over the non-invasive tools but still not capable of capturing subcellular information required for accurate diagnosis of CAD. The highest-resolution *in vivo* imaging modality so far is intravascular OCT, which provides depth-resolved, cross-sectional images of tissue reflectance with an axial (depth) resolution of approximately 10 μm and a lateral resolution of 30–40 μm [17, 18, 21, 22]. It is still very difficult for the existing technologies to identify and quantify cellular content of the plaque cap such as macrophages, cholesterol crystals, smooth muscle cells, collagen and elastin fibers et al, which determines the vulnerability of the plaque in Coronary artery disease. An investigational tool, which can provide sub-cellular information, will greatly improve the detection of vulnerable plaques and therefore saves lives before AMI events.

1.2.5 Micro-optical coherence tomography (μOCT)

Micro-Optical coherence tomography (μOCT), first proposed in [27], is a non-invasive imaging technology for imaging human coronary arteries and respiratory

airways. With the ability to obtain real-time cross-sectional images of tissue pathology at about or below $10\ \mu\text{m}$ level, OCT has been considered as a promising research topic in medical and clinical applications, such as *in vivo* histological diagnosis for detection of precancerous lesions.

Although the standard OCT technology has been used for clinical imaging, it has a resolution of $\sim 10\ \mu\text{m}$, which is too coarse for visualizing most cells. Developments in OCT technologies are in the direction of higher resolution and higher scan rate. Micro-Optical Coherence Tomography (μOCT), an imaging tool for visualizing biological tissue microstructures at the cellular and sub-cellular level has been developed recently [23, 27], demonstrating capability of $1\text{-}2\ \mu\text{m}$ resolution, which is more than one order of magnitude higher than the best existing OCT technology. This breakthrough in spatial resolution brings about a quantum leap in acquired information including cellular and extracellular details that have never been seen in intact human tissue.

The μOCT system achieves $2\ \mu\text{m} \times 2\ \mu\text{m} \times 1\ \mu\text{m}$ resolution (in tissue) at 8 fps [27]. Imaging results from animal and human cadaver tissues establish that μOCT is capable of visualizing unprecedented cellular and subcellular features of human tissue, including endothelium (Figure 1.2 a), leucocytes (Figure 1.2 b-f), platelets and fibrin (Figure 1.2 h), and respiratory cilia (Figure 1.3 and Figure 1.4). Most of the cellular and sub-cellular structures captured by μOCT are self-evident and many of them have been validated by matched medium-power histology.

One-to-one histological validation has been conducted to demonstrate the new information this technology can collect including but not limited to macrophages/foam cells (Figure 1.5 a, b, and d), smooth muscle cells (Figure 1.5 c). The fact that μOCT brings about a quantum leap in acquired information encourages us to continue to improve the spatial resolution towards histopathology *in vivo*. Due to unparalleled resolution and cross-sectional imaging mode of μOCT , new imaging biomarkers for detection of diseases will be discovered and new diagnostic criteria will be developed.

Nuclear morphology is of particular importance for identify types of tumor and determining the stage of neoplastic progression. In human cardiovascular tissue, we discovered that μOCT is capable of visualizing cell nuclei and cytoplasm of leukocytes (Figure 1.2 b-d). μOCT images of leukocyte nuclei demonstrated low image

FIGURE 1.2: μ OCT sub-cellular imaging of coronary arteries. (a) Three-dimensional rendering of the swine coronary artery ex vivo, demonstrating a pattern of raised cells that are consistent with endothelial “pavementing”. (b–h) Human cadaver specimens. (b) Multiple cells that are likely leukocytes (arrows) are seen adhering to the luminal surface in this μ OCT image of a coronary plaque. Two different cell morphologies can be observed, one smaller cell with scant cytoplasm, consistent with a lymphocyte (yellow arrow) and another, slightly larger cell with a highly scattering, abundant cytoplasm, suggestive of a monocyte (green arrow). (c) Cell with an indented, bean-shaped nucleus (green arrow) characteristic of a monocyte. (d) Cell with a multi-lobed nucleus, possibly a neutrophil (blue arrow), is attached to the endothelial surface. (e) Multiple leukocytes tethered to the endothelial surface by linear structures suggestive of pseudopodia (white arrows). (f) Cells with the morphology of monocytes (red arrows) are seen in this cross-section and inset to be transmigrating through the endothelium. (g) Structures consistent with fibrin (magenta arrow) are visible as linear strands bridging a gap in the coronary artery wall. (h) Thrombus (cyan arrow) that appears to contain fibrin, small ($2\text{--}3\text{ }\mu\text{m}$ diameter) highly scattering structures likely to be platelets, and multiple, entrapped cells. Scale bars, $30\text{ }\mu\text{m}$. [27]

FIGURE 1.3: μ OCT image of primary human bronchial epithelial culture and a representative histology. (Top) a μ OCT image of fully differentiated primary human bronchial epithelial (HBE) cells. Cilia are encoded in green based on their beat pattern. Epithelial cells (orange arrow) are readily seen. (Bottom) H&E stained histology of HBE cells. Scale bar: 20 μ m. [26]

FIGURE 1.4: μ OCT images of ciliary motion pattern in HBE culture. A. (top panel) 6-stage schematic of ciliary motion during the recovery stroke; (bottom panel) a μ OCT image of fully differentiated primary HBE cells derived from a normal subject shows cilia tips (green) 3-5 μ m from the apical cell surface, indicating the recovery stroke. Cilia and mucus are presented in pseudo-colors: green and purple respectively. B. (top panel) 4-stage schematic of ciliary motion during the effective stroke; (bottom panel) μ OCT signal of the same cilia after 250 ms that subtend an angle of 114° , delineating an arc with radius of approximately 7 μ m during the effective stroke. C. (top panel) 10-stage schematic of ciliary motion during the full ciliary beat cycle; A time-averaged (4 s) image (bottom panel) shows an arc indicating the effective strokes (yellow arrows) and bilobular pattern of the recovery stroke (orange arrows). [26]

FIGURE 1.5: Validation of μ OCT images using matched histology. Human cadaver specimens. (a) Necrotic core (nc) fibroatheroma with highly scattering lipid-laden macrophages or foam cells (white arrows) infiltrating the cap, also seen in the corresponding histology (upper left inset). An intracellular region of low μ OCT signal, which may represent the nucleus, can be observed within the cytoplasm of some foam cells (e.g. lower left inset, blue arrow). (b) Another lesion, visualized by μ OCT and histology, contains highly scattering foam cells that are ellipsoidal (right insets). (c) Smooth muscle cells by μ OCT appear as spindle-shaped cells (green arrow). Smooth muscle cells producing collagen have a high backscattering interior (right upper inset, yellow arrow) and a “halo” of low backscattering (right upper inset, white arrow). Matching histology (right lower inset) demonstrates that the high backscattering region represents the cell body, while the lower intensity halo corresponds to collagen matrix. (d) Additional macrophages or foam cells (arrows) in necrotic core fibroatheroma.

[27]

intensity, while the cytoplasm had a comparatively high intensity. According to the nuclear morphology, the leukocytes can be further classified as lymphocytes (small cells with scant cytoplasm), monocytes (larger cells with more abundant cytoplasm and bean-shaped nucleus), and neutrophils (multilobulated nucleus) (Figure 1.2 b–d). This finding suggests that it may be possible to use μ OCT to identify atypical cells according to the nuclear morphology.

With the merits of μ OCT mentioned above, μ OCT has been widely used in medical applications to examine specimen in microstructural level. Also, the feature that μ OCT performs in real time allows μ OCT to monitor pathology in high-resolution videos. Real-time diagnosis can also be conducted to facilitate surgical operations.

However, μ OCT is still an *ex vivo* technology and cannot be used for clinical diagnosis as of now. The reasons are 1) μ OCT is still not capable of providing a sufficient depth of field (or depth of focus, DOF) to stably acquire high-resolution images *in vivo*; 2) μ OCT is not able to visualize sub-micron structures which require further improvement in spatial resolution

1.3 Objective and dissertation outlines

This dissertation is dedicated to the development of μ OCT technology, translating it from a lab research setup to a clinical diagnostic tool by providing a clinically viable miniaturized μ OCT endoscopic probe to visualize ciliary function and MCC *in vivo*, improving μ OCT spatial resolutions by implementing a dual-spectrometer configuration and enhancing μ OCT image quality by integrating a novel speckle reduction technique.

One of the objectives of this dissertation is to study the ciliary function and MCC using the benchtop μ OCT technique. This study is presented in Chapter 2, which focuses on cilia regeneration and corresponding function restoration. We performed μ OCT imaging of the recovery of airway after sulfur dioxide injury and quantified the epithelial thickness, cilia coverage, length of cilia, ciliary beat frequency and coordination, and cilia-generated flow over a period up to 52 days. This study shows ciliary function recovered quickly but refined over weeks after injury. It also provides strong evidence that ciliated cells need to align both on the individual level and as a group to function.

Furthermore, with the aim to monitor ciliary function *in vivo*, we dedicated to develop a miniaturized flexible μ OCT endoscopic bronchial probe as presented in Chapter 3. Chapter 3 describes a μ OCT flexible endobronchial probe for clinical translational studies to image mucociliary transport (MCT) in airways of adult and pediatric patients for the purpose of understanding the MCT phenotype of airway diseases such as cystic fibrosis. This is the first μ OCT endoscopic probe that performed airway studies in human to the best of my knowledge. A common-path miniaturized probe prototype was designed and fabricated to provide non-invasive, high-resolution, real-time OCT images *in vivo*. The 2.4 mm diameter and flexibility of the probe allows it to be inserted into the instrument channel of a standard bronchoscope, enabling real-time video guidance of probe placement. Each segment was tested individually to make sure all design requirements were met for clinical application. A laser safety study was conducted before *in vivo* experiment to confirm the tissue safety under broadband, high power laser illumination. *In vivo* experiment with swine showed anatomical structure information of animal airways but quantitative analysis was limited by significant motion artifacts. This flexible endobronchial μ OCT probe was the first step towards clinical μ OCT application and opens possibility to image cilia and mucociliary transport in small distal airways *in vivo*.

The next two chapters are dedicated to improve spatial resolution and image quality of μ OCT based on benchtop systems. These two improvements on the μ OCT benchtop system can be easily translated to or integrated into the flexible endobronchial probe clinical system. In Chapter 4, one-micrometer axial resolution spectral domain optical coherence tomography (OCT) is demonstrated for *in vivo* cellular resolution imaging. It presents a dual-spectrometer ultrahigh resolution μ OCT system using NIR SLD sources that achieved an axial resolution of 1.27 μ m in air. Imaging was conducted on zebra fish larvae to visualize cellular details. Based on its merits in spatial resolution, safety and cost aspects, the proposed technology provides a viable solution for cellular and subcellular resolution imaging.

Chapter 5 demonstrated a MFOCT system for speckle reduction in spectral domain OCT. The speckle-reduced images exhibited better image contrast, signal-to-noise ratio (SNR), contrast-to-noise ratio (CNR), and equivalent number of looks (ENL) compared to the original images without loss of resolution. These advances in

image quality will be particularly useful for visualization of cellular and subcellular structures which suffer most from the speckle noise. A fiber optic multichannel system is better than a free space multichannel system for its ease of construction and maintenance, which also increases its clinical usability.

Chapter 2

In vivo cilia regeneration and ciliary function restoration

This chapter presents a study for *in vivo* cilia regeneration and ciliary function restoration in wild-type adult mice. We performed μ OCT imaging of the recovery of airway after sulfur dioxide injury and quantified epithelial thickness, cilia coverage, length, beating frequency, coordination and cilia-generated flow over a period up to 52 days. Section 2.1 gives a background introduction on the previous study for initiation and maturation of cilia in newborn mice. The study method is introduced in Section 2.2, in which both experimental setup and procedures are described in details. Section 2.3 presents and discusses the experiment results. Section 2.4 summarizes this chapter.

2.1 Background and literature review

Motile cilia are organelles of ciliated epithelial cells that play vital roles in many pathophysiological processes in normal lung, brain, and reproductive systems. Many diseases are associated with defects in cilia motility such as chronic respiratory diseases [29, 30], hydrocephalus [31–35], and infertility [36]. In the respiratory system, cilia move coordinately in both frequency and direction to propel mucus and remove invading particles in the direction from the distal airway to the proximal airway to prevent pulmonary infection. This mechanism is referred to as mucociliary clearance. Francis RJ et al. [37] studied the initiation and maturation

of cilia-generated flow by characterizing the speed, directionality and ciliary beat frequency in newborn and postnatal mouse airways up to postnatal day (PND) 28. The primary finding of their study shows cilia-generated flow stabilized by PND 9 and is directly related to the increasing cilia coverage of the airway epithelium. They also observed a higher ciliary beat frequency, which may relate to respiratory distress at birth. Cilia undergo similar growth process if they are injured. Previous electronmicroscopic studies demonstrated rapid spreading of ciliated cells after naphthalene exposure [38, 39] and ciliated epithelial cells spread and transdifferentiate into distinct epithelial cell types to repair the airway epithelium [40]. Both *in vitro* and *in vivo* models of airway epithelial repair and regeneration were discussed in [41]. But there were limited studies on cilia regeneration. For proper regeneration, not only ciliary structures, but ciliary function should be regenerated on individual level and as a group. In this study, we were the first to monitor the process of re-ciliogenesis after injury with the μ OCT imaging technique.

2.2 Research method

2.2.1 System setup

The μ OCT system is a common-path spectral-domain OCT (SD-OCT) that has an axial resolution of $1.5\mu\text{m}$ and lateral resolution of $2\mu\text{m}$ in tissue, and is the same as the system described in [42]. A broadband supercontinuum light source (SuperK Extreme OCT, NKT Photonics, Birkerød, Denmark) was used to provide light illumination from 600nm to 1800nm with total power of 4W. The light beam passed through a 90:10 broadband beam splitter (BS029, Thorlabs Inc.) to both limit the optical power to below 40 mW and the wavelength range to 600 nm – 1000 nm. The remaining light illumination was focused by an achromatic lens (AC050-010-B, Thorlabs Inc.) and coupled into a single mode fiber (SM600, Thorlabs Inc) to a benchtop probe. A 45 degree rod mirror (54-092, EdmundOptics) separated the reference and sample arms and introduced a circular obscuration in the center of sample arm for a balance of lateral resolution and depth of focus. The reference and sample signals recombined at the 45 degree rod mirror and 90% of the returning optical power was delivered to the μ OCT spectrometer. The spectrometer consisted of a grating (Wasatch 940lp/mm @830nm), an $f=85$ mm focusing lens

FIGURE 2.1: μ OCT system schematic for cilia regeneration study[42]

(Nikon, Japan) and a line-scan camera (SPL4096-140k, Basler, Germany). Imaging was conducted at 20 kHz A-line rate.

2.2.2 Experiment procedures

Tissue preparation was conducted by Dr. Vladimir Vinarsky from Pulmonary & Critical Care Medicine department of Massachusetts General Hospital. The author performed μ OCT imaging, data collection and analysis in this experiment.

8-week-old wild-type mice were exposed to sulfur dioxide for 4 hours until the cilia and epithelium in their airways were eliminated. The airway recovery were observed through day 1 to day 52 using μ OCT imaging technique. In these experiments, three mice were sacrificed by euthanasia via compressed CO_2 in closed chamber at each time point and their trachea were excised. We cut each trachea into the left half and the right half and chose left half from day 6 to day 52 for consistency. Excised trachea were imaged *in vitro* under benchtop μ OCT system using standard procedures as shown in Figure 2.1. The regeneration progress was analyzed based on the epithelial thickness, cilia coverage, length, beating frequency, coordination

and generated flow with the help of μ OCT imaging at 80 frames per second (fps) up to 52 days. For epithelial thickness, cilia coverage, length, beating frequency and coordination analysis, five sets of data at different locations with $100\mu\text{m}$ spacing were recorded. Results were taken as an average across the five sets of data. To quantify the cilia-generated flow, we opened up the tracheal lumen and imaged the respiratory mucosa. We placed $7\mu\text{l}$ polystyrene-based microsphere solution of concentration $3.64 \times 10^8 \text{ Particles/ml}$ into the trachea lumen and tracked the speed of the microsphere flow. The microspheres were $0.5\mu\text{m}$ in diameter. μ OCT imaging sessions were conducted at 10 different locations across $500\mu\text{m}$ range with $50\mu\text{m}$ spacing with scan direction from the proximal end to the distal end. Similarly, another 10 sessions were conducted with orthogonal scan direction, from left to right. Speed was calculated as the absolute value of the vector sum of the velocities obtained in these two orthogonal directions respectively. For consistency purpose, trachea left half ring 4 -5 was selected throughout all imaging sessions.

2.3 Imaging results and discussion

We categorized the procedures of cilia recovery into two phases. Phase I - regeneration – the development of ciliated cells and growth of cilia to adequate coverage and length to support mucociliary clearance transport, and phase II - refinement – the cilia to improve alignment and beat coordination and increase the rate of mucociliary transport.

2.3.1 Regeneration of ciliated epithelial cells: day 1 - day 5

Ciliated cells were completely eliminated after sulfur dioxide exposure. The epithelium was $10 - 20 \mu\text{m}$ in thickness after injury as shown in Figure 2.2. The epithelium thickness increased during *in vivo* regeneration from day 1 to day 5 and restored to its normal value of around $45\mu\text{m}$ at day 5 (Figure 2.3). We also observed that at day 5, the epithelium surface was fully covered with ciliated cells whereas cilia were slightly shorter ($6\mu\text{m} - 7\mu\text{m}$) than normal ($9\mu\text{m} - 10 \mu\text{m}$) and not functioning yet. Cilia were fully recovered and start functioning by day 6 (Figure 2.4).

FIGURE 2.2: Images showing epithelium thickness increase from day 1 to day 5 after injury

FIGURE 2.3: Figure of cilia length from day 5 to day 24 showing cilia regenerated to normal length at day 6 after injury

FIGURE 2.4: Figure of cilia coverage from day 5 to day 24 showing epithelium surface was more than 90% covered with ciliated cells at day 6 after injury

FIGURE 2.5: Histogram of cilia-generated flow from day 7 to day 52. Day 7 to day 15: cilia-generated flow speed increased significantly. Day 15 to day 52: cilia-generated flow speed was stabilized.

2.3.2 Refinement of coordinated cilia functions: day 6 – day 15

We find the cilia-generated flow started at day 6 with increasing speed gradually throughout day 6 to day 15. We referred to this stage of injured airway recovery as the refinement phase. Refinement continues over weeks and months with much slower rate after day 15. Figure 2.5 shows the histogram of the tracheal flow speed. The distribution of speeds broadened over time and shifted to higher value.

Notably, a significant spike in CBF was observed at day 6, being $29\text{Hz} \pm 1.5\text{ Hz}$ and reduced to $23\text{ Hz} \pm 2.5\text{ Hz}$ in day 7 (Figure 2.6). This significant spike in CBF when the cilia had just grew to its full length comparing to all other time points was coincide with the observation for newborn mouse airway development by [37].

We noticed that from day 6 to day 15, there was little change in epithelium thickness, cilia coverage, length and beat frequency. Figure 2.7 compares these parameters between day 7 and day 15. It can be observed that both epithelial was fully covered with cilia of similar length at similar beat frequency. However, the average cilia-generated flow increased significantly from around $9.8\text{ }\mu\text{m/s}$ to $36\text{ }\mu\text{m/s}$ from day 7 to day 15 (Figure 2.9 and Figure 2.5).

FIGURE 2.6: Figure of CBF from day 6 to day 52 showing a significant spike at day 6

FIGURE 2.7: μ OCT images obtained from day 7 and day 15 showing similar cilia length and coverage

FIGURE 2.8: Images of cilia frequency correlation showing a stronger cilia beating coordination in day 15. The brightness indicates how strong their beat frequency correlated with each other and the color represents the ciliary beat phase.

FIGURE 2.9: Figure of the speed of cilia-generated flow from day 6 to day 52 showing significant speed increase from day 6 to day 15

FIGURE 2.10: Figure of the ciliary beat frequency correlation from day 6 to day 52 showing significant ciliary beat frequency correlation increased from day 6 to day 15

We hypothesized that the increase in cilia-generated flow was the result of increasing coordination between cilia movement, which means more aligned cilia beating with their neighbors. To test this hypothesis, we quantified the beat coordination by computing frequency correlation of 10 selected cilia. The frequency correlation was defined as the cross-correlation of the frequency spectrum of the cilia. We confirmed an increasing coordination from day 6 to day 15, matching with the speed increasing (Figure 2.9 and Figure 2.10). Figure 2.8 shows the frequency correlation images of day 7 and day 15 respectively. The brightness indicates how strong their beat frequency correlated with each other and the color represents the ciliary beat phase. Although in both days, the epithelial had comparable epithelium thickness, cilia coverage and beat frequency, the brighter region at day 15 (Figure 2.8) indicates a stronger ciliary beat coordination, which we speculated to be the reason for the difference in cilia-generated flow speed.

2.3.3 Experimental verification of *in vitro* regeneration

In order to rule out the factor of sample differences, we further conducted *in vitro* experiment by checking refinement in the same tissue at the same location over time and confirmed the findings. Two mice were sacrificed at the first time point and the tracheas were excised. Using the same procedures, we cut the trachea into two parts longitudinally and imaged the left part only. Tissue samples were kept in an incubator at 37 degrees Celsius and 5% O₂ and 95% CO₂ concentration between two imaging sessions. We imaged the same location by finding the center of the fourth trachea ring counting from the proximal end longitudinally. Same

FIGURE 2.11: Figure of the ciliary beat frequency from day 6 to day 9 for *in vitro* regeneration. Green and blue lines represent two different samples.

FIGURE 2.12: Figure of cilia-generated flow speed from day 6 to day 9 for *in vitro* regeneration. Green and blue lines represent two different samples.

FIGURE 2.13: Figure of the ciliary beat frequency correlation from day 6 to day 9 for *in vitro* regeneration. Green and blue lines represent two different samples.

experimental setup was used as shown in Figure 2.1 We observed CBF had a spike around day 6 – 7 and start decreasing (Figure 2.11), whereas speed kept increasing through day 6 to day 9 (Figure 2.12) in line with an increasing cilia coordination (Figure 2.13).

2.4 Summary and outlook

In brief, we performed μ OCT imaging of the recovery of adult mouse airway after sulfur dioxide injury and quantified epithelium thickness, cilia coverage, cilia length, cilia beat frequency and coordination, and cilia-generated flow over a period up to 52 days. In this study, we categorized the injured airway recovery into two phases. Day 1 to day 5 was referred as the regeneration phase, when the epithelium became thicker and ciliated cells were divided from basal cells. By day 5, epithelium surface was fully covered with ciliated cell. Cilia were slightly shorter and had not started functioning yet. Cilia started to function at day 6 and generate effective flow. Therefore, day 6 to day 15 was referred as the refinement phase, when both speed and ciliary beat coordination increased over time with little change in CBF.

An overshoot in CBF was observed at the late regeneration phase or early refinement phase. It could be a sign of lag in cilia coordination at the early stage of regeneration. Future study could be conducted to find out more about the relationship between CBF and cilia coordination.

Chapter 3

μ OCT flexible endobronchial probe toward clinical applications

This chapter presents a μ OCT flexible endobronchial probe for clinical translational studies to image mucociliary transport (MCT) in airways of adult patients for the purpose of understanding the MCT phenotype of airway diseases such as cystic fibrosis. A common-path miniaturized probe was designed and fabricated to provide non-invasive, high-resolution, real-time OCT images *in vivo*. Section 3.1 gives a background introduction on the clinical needs for such imaging tool and the literature review on OCT endoscopic probes. Section 3.2 provides an overview of the μ OCT flexible endobronchial probe, which can be segmented into three main parts: optical core, protective sheath, and ergonomics. It also describes electrical and optical safety considerations. Section 3.3, Section 3.4, and Section 3.5 describe the design, fabrication and performance test results of each segment of the μ OCT flexible endobronchial probe system in detail. *Ex vivo* tissue imaging experiment using mouse trachea as sample was conducted to validate all design requirements were met, verify beating cilia could be visualized and confirm MCT dynamics functions could be quantified. This is discussed in Section 3.6. Section 3.7 presents a laser safety study to demonstrate the proposed study equipment and operation procedures have no potential safety hazard to biological tissues prior to use in human patients. The first μ OCT flexible probe *in vivo* experiment with swine model was conducted and discussed in Section 3.8. Finally, Section 3.9 summarizes this chapter.

3.1 Background and literature review

The recent development of μ OCT imaging technique has further improved resolution in both axial and lateral directions by an order of magnitude compared to traditional techniques, allowing cellular and sub-cellular microstructures of biological tissues to be resolved with applications in a variety of organ systems [25, 27, 43]. In particular, OCT has significant potential in the assessment of lung disease. [44] conducted *in vivo* OCT imaging in the swine airways and mapped the OCT images to histology images to verify the pathological structures. OCT could also see beyond bronchoscope for disease diagnosis [45]. In addition, *In situ* imaging of lung alveoli with an OCT needle probe was conducted in [46]. Our group has applied μ OCT to the dynamic imaging of respiratory cilia [23, 24, 26, 42], the organelles on airway epithelia that are responsible for mucus clearance. Clinical translation of μ OCT so that it can be used for diagnostic imaging *in vivo* requires miniaturized probes that are small, stable and flexible to reach a targeted area of interest. Much research has been devoted to high resolution OCT probe development [47–53]. In the past decade, Alexandre R. Tumlinson et al. had developed a FD-OCT endoscopic common-path probe of 2 mm diameter and conducted *in vivo* experiment with mouse colon, visualizing colonic mucosa, muscular mucosa, submucosa, muscularis externa, and serosa layers [49]. The axial resolution was $\sim 4 \mu\text{m}$ in air. In addition, Jiefeng Xi et al further improved the resolution to $\sim 3 \mu\text{m}$ axial and $6.2 \mu\text{m}$ in air, without a common-path configuration [53]. The optical performance of this probe was verified by imaging guinea pig esophagus *in vivo* with three dimensional full-circumferential scan, visualizing stratum corneum, epithelium, lamina propria, muscularis mucosae, submucosa and muscularis propria layers. Yet, their resolutions were still not high enough to resolve cilia and the scanning mechanisms were not designed to study mucociliary clearance dynamic functions. In a nutshell, the clinical requirements for respiratory systems studies were unmet.

Our lab has previously developed a first generation common-path μ OCT probe [42]. It used a gold coated prism along the long side with a circular opening in the center to split the input beam into reference arm and sample arm, thus reference arm passed straight through while sample arm was reflected 90 degrees to form side-view configuration. There are three major limitations associated with this design. Firstly, the probe used 2 mm diameter optics, and after adding driveshaft and protection sheath, the final probe diameter was 4 mm. Simultaneous navigation

using standard bronchoscope is difficult since it is too big to fit into the 2.8mm instrument channel. Secondly, a rigid driveshaft was chosen for ease of motion translation from proximal end piezo scanner to distal end optics. The first generation μ OCT probe is limited to image trachea of lower respiratory system, but access to smaller bronchia was practically impossible. Lastly, the reference reflector was placed by means of a set screw, which was not stable enough and moved during experiment. The experiment suffered from significant image degradation and occasional loss of images. We also reported another μ OCT probe design featuring significantly reduced diameter and increased flexibility, though this configuration requires a short working distance that is incompatible with imaging in relatively large airways [54].

In a nutshell, a novel μ OCT probe is desirable with smaller size, more stable reference reflector positioning and better navigation control.

The μ OCT probe described herein resolved all the limitations mentioned above.

3.2 Probe module overview

3.2.1 Probe introduction

The μ OCT flexible endobronchial probe is designed to be introduced into patients through a bronchoscope instrument channel to perform μ OCT imaging of respiratory epithelium. The probe transmits optical signal from and to the μ OCT console and contains miniaturized optics, including a borosilicate spacer, a GRIN lens and a reference reflector, for μ OCT beam formation and interferometry. A protective sheath is employed to enclose the probe optics to physically protect the epithelium from the imaging apparatus. An ergonomic component is employed to insert the probe into the instrument channel of a commercial bronchoscope as well as manipulate the probe during operation. At last, a piezo scanner, which is enclosed in the handle container, is used to scan the probe optics inside the stationary protections (Figure 3.1). All of the components are described in the following sections in detail.

FIGURE 3.1: μ OCT probe module overview

The probe is also powered by the μ OCT console (Section 3.8). The interface is comprised of two electrical connections and one optical connection. The bronchoscope provides visual guidance and navigation for placement of the probe imaging optics over a target region.

3.2.2 μ OCT airway imaging procedures

The procedures begin by attaching the ergonomic probe adapter assembly to the working channel port of a standard commercial bronchoscope. The μ OCT optical probe core is fed inside a disposable flexible protective sheath and fixed in place in the probe insertion assembly. The entire probe is then fed through the probe adapter assembly into the bronchoscope working channel until it is just flush with the distal face of the bronchoscope.

Once in place, standard flexible bronchoscopy procedures are carried out to identify regions of the airway suitable for μ OCT imaging. A physician trained in the operation of a flexible video bronchoscope places the instrument into the airway with real-time video guidance and navigates the bronchoscope to a desired μ OCT imaging target. Upon visualizing the target region for μ OCT imaging, the μ OCT probe is advanced through the working channel of the bronchoscope under video guidance. When the distal end of the probe is in proximity to the target site, the

thumb screw on the ergonomic adapter is tightened and the probe is fixed in place. The design of the ergonomic adapter insertion tube prevents insertion of the probe into the airway beyond bronchoscope up to a maximum of 10 cm. The μ OCT imaging system is then enabled, which activates laser illumination and mechanical scanning. Each μ OCT imaging sequence is of approximately 5 seconds in duration. The bronchoscope navigation itself will be used to assist in the placement of the μ OCT probe relative to the desired target. Visual contact with the probe tip will be maintained at all times through the bronchoscope video signal.

Upon completion of imaging, the μ OCT probe tip will be retracted into the bronchoscope instrument channel. The probe can then be removed by retracting it fully from the bronchoscope while the bronchoscope remains placed in the airway. Alternatively, the bronchoscope itself may be retracted from the patient at the termination of the procedures with the μ OCT probe contained in the instrument channel, but with the probe tip retracted partially so that no portion of the μ OCT probe protrudes from the bronchoscope.

3.2.3 Electrical safety

Electrical power and control signals are sent to the linear motor housed in the ergonomic grip. Several safety features are included in the design to minimize risk of electrical shock to the patient or operator during normal use and servicing. First, the electrical power to the motor is generated from an electrical isolation transformer. The piezoelectric motor is housed in a water-proof housing made of electrically insulating plastic material. The electrical components are double-insulated, and all electrical cabling is transmitted to the ergonomic segment through a water-proof flexible umbilical tube. The materials that comprise the flexible probe itself are all electrical insulators and cannot convey current to the distal portion of the probe that is in contact with the patient airway. The probe is subject to lead leakage testing according to IEC 60601-1 to cardiac floating lead standards, with a maximum permissible patient-to-ground, operator-to-ground, and patient-to-operator current leakage of 10 μ A under normal conditions 50 μ A under single fault conditions.

3.2.4 Laser safety

The major risks were unintended exposure or excessive exposure to laser radiation to the patient or operator. To mitigate these risks μ OCT imaging was limited to imaging segments of 30 seconds duration and only occurred during coordinated action of the system operator and clinician to minimize risk of unwanted laser exposure. When three conditions were met,

1. the clinician's footswitch was engaged
2. the operator's switch was engaged
3. the optical power meter measured a beam of light that was within a safe range, the supercontinuum laser source was activated and entered the console optical subsystem

In the optical design of μ OCT console, both the optical spectral filter set and 70:30 beam splitter ensured that the total laser power that reaches the distal end of the probe does not exceed 40 mW. Laser exposure at the tissue at any one instance was limited to 30 seconds with the use of a programmable shutter placed between the beam splitter and the proximal end of the probe. This corresponds to an estimated maximum tissue radiant exposure to be 0.008 J/cm^2 , below the ANSI standard level for skin exposure. The shutter could also be manually closed by the operator before the end of the 30s time period. Laser emission and shutter activation signals from the clinician and operator, respectively, had to be re-activated to resume imaging of the next tissue location. The power readout of optical power meter was continuously monitored in the software. In the event that the optical power meter reading deviates from the expected by a threshold, the laser interlock would trip and disable the laser emission immediately. Detail laser safety test was performed to ensure there is no tissue damage hazard at intended optical power of 20 mW for a period of 30 seconds and presented in Section [3.7](#).

PARAMETER OF INTEREST	SIZE / FREQUENCY	TECHNICAL REQUIREMENTS
CBF	4 Hz to 12 Hz (Human) 11 Hz to 17 Hz (Swine)	Frame rate ≥ 24 Hz Frame rate ≥ 34 Hz
PCL	$5\ \mu\text{m}$ to $7\ \mu\text{m}$	Axial resolution $\leq 2.5\ \mu\text{m}$
ASL	$10\ \mu\text{m}$ to $20\ \mu\text{m}$	Axial resolution $\leq 5\ \mu\text{m}$

TABLE 3.1: Summary of parameters of interest of mucociliary functions and design requirement

3.3 Probe optics

3.3.1 Optical design

3.3.1.1 Design requirement

The μ OCT probe presented herein integrated both reference arm and sample into a common-path configuration to minimize the material dispersion and ensure optimum optical quality. Comparing to conventional OCT probes, the μ OCT probe has an order of magnitude better resolution, thus it is more susceptible to dispersion. Technical performance is the primary design objective. The device should be able to quantitatively assess airway surface liquid (ASL) depth, periciliary layer (PCL) depth and ciliary beat frequency (CBF). Table 3.1 summarizes the technical design requirement in order to visualize mucociliary functions.

In this study, we chose swine model because swine lower respiratory system anatomically resembles human lower respiratory system and the existence of generic CF swine opens possibilities for future studies of CF pathophysiology. According to Nyquist-Shannon sampling theorem, the frame rate should be at least twice as fast as the CBF to reliably capture cilia beating dynamics and be as fast as possible to mitigate motion artifacts. Thus frame rate was chosen to be 40 Hz. As the cilia length is around $5\ \mu\text{m}$ to $7\ \mu\text{m}$, the axial resolution should be at least $2.5\ \mu\text{m}$ in order to resolve cilia and mucociliary transport. The sensitivity should be at least 80 dB, which was chosen to be the sensitivity when it is barely enough to visualize cilia at the top most layer. The depth-of-focus should be at least $200\ \mu\text{m}$ and the lateral field of view should be at least $300\ \mu\text{m}$ in order to visualize multi-layered environment of mucus, cilia and epithelium. Performance requirements were listed in Table 3.2.

REQUIREMENTS	INPUTS	POTENTIAL SOLUTION
Resolve cilia, mucociliary transport	Axial resolution $\leq 2.5 \mu\text{m}$	Laser: NKT Photonics SuperK Extreme OCT with wavelength bandwidth 650 nm - 950 nm
	Lateral resolution $\leq 5 \mu\text{m}$	NA > 0.1
	Sensitivity $\geq 80 \text{ dB}$	Less than -6 dB loss from fiber input to sample
	Minimize dispersion	Common path probe design
Visualize multi-layered environment of mucus, cilia, epithelium	depth of field $\geq 200 \mu\text{m}$	Centered annular beam apodization of approximately $\frac{1}{2}$ max NA
	Spectrometer ranging depth $\geq 200 \mu\text{m}$	Spectrometer axial scan range $\geq 200 \mu\text{m}$ at less than -3 dB sensitivity rolloff
	Working distance $\geq$ radius of the outer most sheath	Focal distance of 2 mm after GRIN lens.
	Lateral field of view $\geq 300 \mu\text{m}$	Scanner: PI PZT scanner $\sim 500 \mu\text{m}$ lateral FOV unloaded
Capture cilia beating dynamics	Frame rate $\geq 34 \text{ Hz}$	40 fps imaging
	Repeatable interframe imaging, variation < 1 ciliary beat area	Driveshaft material: PEEK tubing and interframe lateral A-line drift position $< 2 \mu\text{m}$ RMS

TABLE 3.2: Design input for optical parameters

3.3.1.2 Zemax simulation

The optical core of the probe was a common-path, circular-obscuration apodization design similar to [42]. The common path design minimized material dispersion, and the beam apodization extended DOF as reported in [27].

Zemax simulation for sample arm optics was conducted to determine the length of each optical component (Figure 3.2). Based on the resolution and working distance requirements, the GRIN lens NA was theoretically predicted to be > 0.1 , thus the spot size exiting GRIN lens should be around $500 \mu\text{m}$ in diameter. The spacer length was determined to be $2.7 \text{ mm} \pm 100 \mu\text{m}$ to ensure the beam was sufficiently expanded to achieve high resolution. The outer diameter of the GRIN lens is 1mm and the input beam size before the GRIN lens is around $550 \mu\text{m}$ diameter. As there was a discrepancy between the simulation results and the experimental

FIGURE 3.2: μ OCT probe optics sample arm Zemax simulation

results, several iterations of fabrication and performance tests were conducted to adjust simulation parameters. The GRIN lens length was finally set to $1.4 \text{ mm} \pm 30 \text{ }\mu\text{m}$ to achieve focal distance of 2 mm . GRIN lens length had much tighter tolerance because its focusing power is very sensitive to the optics length. As standard borosilicate fiber ferrule was designed to fit optical fibers inside and had inner diameter of around $129 \text{ }\mu\text{m}$, the center obscuration size was set to $129 \text{ }\mu\text{m}$.

Resolution analysis

The laser source had a bandwidth from 650nm to 950nm , centering at around 810nm . We set 810nm as the primary wavelength weighted as 1 and both 650 nm and 950 nm weighted as 0.5. The predicted Airy disk radius was $4.56 \text{ }\mu\text{m}$ (Figure 3.3). The lateral resolution, defined by the FWHM of the focused spot, was predicted to be $3.84 \text{ }\mu\text{m}$.

The FWHM of PSF at focus was predicted to be around $4 \text{ }\mu\text{m}$ (Figure 3.4).

Depth of field analysis

Depth of field was extended as the total effect of chromatic focal shift and beam apodization. From the through focus spot diagram (Figure 3.5), the geometric

FIGURE 3.3: μOCT probe optics sample arm Zemax simulation spot diagram at focus

FIGURE 3.4: μOCT probe optics sample arm Zemax simulation point spread function at focus

FIGURE 3.5: μ OCT probe optics sample arm Zemax simulation focus spot diagram

radius would be doubled at $80\ \mu\text{m}$ away from the focus. Zemax predicted the chromatic focal shift to be $120\ \mu\text{m}$ between 650nm and 950nm (Figure 3.6) and Matlab predicted the depth of field to be extended by 17% with 50% center obscuration (Figure 3.7). The Matlab model was derived from Gaussian beam propagation. Optical properties and propagation length for difference waveguides, GRIN, spacer, and air were defined separately. The incident beam field distribution was simulated along propagation. The depth of focus was the field distribution along the depth on axis at focus.

3.3.2 Fabrication

The μ OCT flexible bronchial probe fiber-optic core delivers laser light from the μ OCT console to the bronchial epithelium and transmits reflected light from the tissue back to the μ OCT console. The following describes the illumination path through the probe optics, with collection occurring in reverse (Figure 3.8).

Laser light was transmitted to the probe through a SM600 fiber (Thorlabs. Inc.), expanded within a borosilicate spacer (Fisher Scientific, 1mm Diameter), and focused by a 1.0 mm GRIN lens (Edmund Optics, 1.0 mm diameter, 810 nm DWL, 0.0 mm WD, NIR Coated). The converging light then encountered the 41 degrees

FIGURE 3.6: μOCT probe optics sample arm Zemax simulation chromatic focal shift diagram

FIGURE 3.7: μOCT probe optics sample arm Matlab simulation for depth of field

FIGURE 3.8: Illustration of μOCT endoscopic probe distal end beam propagation

FIGURE 3.9: μ OCT endoscopic probe distal end optics components schematic. 1: Outer sheath, 2: Driveshaft, 3: Fiber, 4: Ferrule, 5: Spacer, 6: GRIN lens, 7: Sample reflector, 8: Reference reflector. (Table 3.3)

μ OCT BRONCHIAL PROBE OPTICAL SUBSYSTEM	
1 – Outer sheath	5 – Spacer
2 – Driveshaft	6 – Grin Lens
3 – Fiber	7 – Sample Reflector
4 – Ferrule	8 – Reference Reflector

TABLE 3.3: Components list of μ OCT bronchial probe optical subsystem

polished face of a hollow glass cylinder (1 mm OD, 129 μ m ID). The polished ferrule face was gold-coated to promote reflectivity, forming an elongated annular reflector. A portion of the beam reflected by the annular coating became the OCT sample beam and was directed out of the imaging window to the sample. The slight acute angle of reflection from the ferrule served to reduce specular reflections from the window and tissue surfaces. A portion of the beam entering the hollow ferrule center became the OCT reference, reflected from a cleaved fiber acting as a reference reflector (Figure 3.8). The cleaved fiber was not coated; the relatively low 4% reflectance from the surface returned sufficient power to act as the OCT reference while maintaining high sensitivity. The space after the spacer remains open and both reference beam and sample beam propagate in air after GRIN lens. Index-matching UV-curing optical adhesive (Norland, NOA 86) was used to bond all optical components. Compared to our previous beam apodization method [42], involving manual removal of reflective coating from a reflective prism, the ferrule-based method greatly improved beam shape accuracy and centration, simplifying fabrication, and significantly lowering the cost.

A stainless steel hypotube (McMaster-Carr, 18 Gauge) connected the ferrule reflector to the GRIN lens (Figure 3.10). The total rigid length of the 1 mm diameter distal optics was approximately 9 mm (Figure 3.10). The entire optical subsystem

FIGURE 3.10: Illustration of μ OCT endoscopic probe distal end fabrication of bare optics: Optics 1mm in diameter and less than 9mm in length

was protected by a stainless steel hypotube (VitaNeedle, 1/16 OD X.003) with a slot milled into the side to allow light transmission (Figure 3.11). The probe optics were affixed to the driveshaft via the protective hypotube.

The probe optics was attached to the distal end of a flexible tube called the driveshaft. The driveshaft is further contained inside a disposable, flexible over-tube called the protective sheath (discussed in Section 3.4). The driveshaft was configured to slide back and forth inside the protective sheath, allowing scanning of the optical beam focus across the epithelium while the driveshaft was held safely inside the sheath. Two coaxial flexible tubes were needed to support longitudinal scanning, with the outer sheath remaining static relative to the tissue and bronchoscope, and the driveshaft transmitting the motion of the linear motor to the distal optics. The means to push and pull the driveshaft are described in Section 3.5.

3.3.3 Performance test

Optical tests were performed to ensure the resolution, sensitivity, working distance, etc., meet design requirements listed in Section 3.3.1.1 to resolve airway cilia.

FIGURE 3.11: Illustration of μ OCT endoscopic probe distal end fabrication after putting protection hypotube

3.3.3.1 Test setup

The probe was connected to an imaging platform that included the SD-OCT spectrometer, drive electronics, data acquisition hardware, and computer system (Figure 3.12). The light source was a supercontinuum laser (SuperK Extreme OCT, NKT Photonics, Birkerød, Denmark). The laser illumination was passed through an NKT customized spectral filter set and 90:10 broadband beam splitter, and was coupled into the probe fiber, with a resultant 18 mW optical power delivered to the sample. Imaging was conducted at 35 kHz A-line rate (27 μ s integration time). The spectrometer consists of a grating (Wasatch 1200lp/mm @830nm), a customized focusing lens and a line-scan camera (SPL8192, Basler, Germany).

3.3.3.2 Test scheme

A list of the non-destructive optical qualification tests performed is summarized in Table 3.4. These tests were to verify the fabricated μ OCT probe could meet design requirements for mucociliary function studies.

	TEST NAME	TESTED ITEM	PASS CRITERIA
Optical Qualification Tests - Non-Destructive	Lateral resolution	Assembled optical subsystem	$< 4\mu\text{m}$ in aqueous medium
	Axial resolution	Assembled optical subsystem	$< 2\mu\text{m}$ in aqueous medium
	Throughput	Assembled optical subsystem	Light throughput is over 15% to sample arm
	Spot distortion and concentricity	Optical probe subsystem	Observed annular shape with concentric apodization size approximately half of total beam size
	Reference intensity	Optical probe subsystem	The peak reference power should be between $2\mu\text{W}$ and $3\mu\text{W}$.
	Back Reflections	Optical probe subsystem, before reference attachment	Back reflections less than -2 dB of standard reference power
	Focal distance	Optical probe subsystem	Focal point is at outside of imaging window
	Imaging window optical transparency	Imaging window	Throughput higher than 75%
	EtO (ethylene oxide)	Assembled sheath	No evidence of visual defects in beam output profile, materials and interfaces. Optical power drop $< 10\%$
	Image check	Assembled probe	Solid beads phantom of $1\mu\text{m}$ to $50\mu\text{m}$ size, Minimum beads size can be seen of less than $5\mu\text{m}$ lateral and less than $2\mu\text{m}$ axial. Reference arm pathlength distance is around 200 - 400 μm shorter than sample arm pathlength distance.

TABLE 3.4: Non-destructive optical qualification test scheme

FIGURE 3.12: Schematic diagram for the μ OCT system and flexible endobronchial probe

FIGURE 3.13: Optical test result of lateral resolution

3.3.3.3 Lateral resolution

The lateral resolution was measured using as resolution target (200 lp/mm Ronchi Ruling, Edmund Optics) on an automatic scanning stage. Test result showed the probe was able to resolve two adjacent bars on the resolution target of $5\mu\text{m}$ spacing and above (Figure 3.13).

FIGURE 3.14: Optical test result of axial resolution

3.3.3.4 Axial resolution

Axial resolution was measured by putting a glass slide on the automatic stage and plotting the full-width-half-maximum (FWHM) of the point spread function of one A-line. Test result showed the PSF of an A-line had FWHM of $1.8 \mu\text{m}$ in air (Figure 3.14), estimated $1.35 \mu\text{m}$ in aqueous medium.

3.3.3.5 Throughput

We measured the optical power of the input light beam and that of the endo-bronchial probe sample arm. Probe throughput was calculated to be the ratio of the optical power between the sample light beam and the input light beam. Test results were summarized in the Table 3.5. An average of more than 20% throughput was achieved. Around 50% of the optical power was lost inside the optical fiber due to non-linearity effect for broad wavelength band.

3.3.3.6 Spot distortion and concentricity

We visually inspected the beam profile to be annular shape. The apodization was concentric and the center obscured hole size was approximately half of the total

Initial power 83mW	Probe 1	Probe 2	Probe 3	Probe 4	Probe 5
Before Reference Split (mW)	47.5	53	46	44	40
After Reference Split (mW)	20	21.2	18	19	16.3
Throughput (%)	24	22.5	21.7	22.9	19.6

TABLE 3.5: Table of μ OCT bronchial probe throughput

FIGURE 3.15: Optical test result of spot profile

beam size (Figure 3.15).

3.3.3.7 Reference intensity

High and stable reference intensity is essential in OCT imaging technique to achieve good sensitivity. In this probe design, we used cleaved fiber terminal as reference reflector. The 4% reflectivity provided more than enough power to saturate the spectrometer without any reflectivity-increasing coating. Thus the reference reflector angle was slightly tilted during fabrication to adjust the reference power to be around half of the camera dynamic range. We checked the peak reference power to be around 3100 shown on the μ OCT console software (on 0 to 4096 scale) with FWHM of around 1300 pixels (Figure 3.16).

FIGURE 3.16: Optical test result of reference spectrum

FIGURE 3.17: Optical test result of back reflection spectrum

3.3.3.8 Back reflection

High unwanted back reflection decreases camera dynamic range which reduces μ OCT imaging system sensitivity. During fabrication, we also checked probe back reflection using μ OCT software before attaching GRIN lens. The peak back reflected power was less than 600 (on 0 to 4096 scale) (Figure 3.17), corresponding to less than -7dB of standard reference power.

FIGURE 3.18: Optical test result of focal distance. The imaging window was shown as two horizontal lines with impurities in between. The focus was identified as the position where the trachea image had the best resolution.

3.3.3.9 Focal distance

Focal distance had to be tested after threading the probe through the full assembled outer sheath (will be described in details in Section 3.4). We put a piece of mouse trachea against the imaging window and checked the image position using μ OCT console software. Focused image was shown outside of the imaging window (Figure 3.18).

ITEM	PROFILE BEFORE ETO	PROFILE AFTER ETO	POWER BEFORE ETO	POWER AFTER ETO
Probe 1			9.2 mW	9.0 mW
Probe 2			9.3 mW	9.0 mW
Sheath 1			7.8 mW throughput	7.5 mW throughput

TABLE 3.6: EtO sterilization test results for probe optical performance

3.3.3.10 Imaging window optical transparency

We measured the optical power of the sample beam from the endobronchial probe before and after the imaging window and took the ratio as the imaging window throughput. The imaging window throughput was 85%. Detailed test results were presented in Table 3.6.

3.3.3.11 EtO (ethylene oxide) sterilization

An assembled sheath was examined before and after EtO sterilization for optical and mechanical properties. EtO sterilization had negligible influence on the optical parameters of both inner optics and imaging window (Table 3.6).

3.3.3.12 Image check

To verify the axial resolution and depth of field of the probe's optics, we imaged a solid bead phantom composed of a mixture of 30-50 μm diameter poly dispersed glass spheres, 10 μm polystyrene mono dispersed polystyrene spheres, and diatomaceous earth mixed in the range 1 - 3 μm in size with various forms in a matrix of Sylgard 184 elastomer. We put solid beads phantom on an automatic scanning

FIGURE 3.19: Optical test result of solid beads phantom

stage and adjust the solid beads phantom position to be at the focus of the probe. Upright image was shown on the software at around $300\ \mu\text{m}$ away from the DC position, which indicated the reference reflector was $300\ \mu\text{m}$ shorter than focal distance. Minimum beads size was measured to be $4\ \mu\text{m}$ lateral and $1.5\ \mu\text{m}$ axial, confirming the results of resolution tests (Figure 3.19).

3.4 Probe sheath

3.4.1 Mechanical design requirements

The total diameter of outer protective sheath should be less than $2.8\ \text{mm}$ in order to fit into a bronchoscope instrument channel and total length should be more than $80\ \text{cm}$ in order to reach the region of interest (2^{nd} generation bronchia). The sheath was intended to stay relative stable to the tissue of interest to avoid motion artifacts as much as possible whereas having sufficient working distance to allow

REQUIREMENTS	INPUTS	POTENTIAL SOLUTION
Investigate mucociliary transport phenotype in small airways	Must reach 2 nd generation airways	Outer tube = 2.4mm Optical transparent imaging window: Iridium flexible heat shrink tubing - Engineered and coated driveshaft outer surface
Compatible with video bronchoscope instrument channel	Flexible, outer diameter < 2.5 mm, length > 80 cm	Test: Insertion into Olympus BH-1TH190 Outer tube = 2.4 mm OD Outer tube tip to hilt length > 80 cm
Accurate knowledge of μ OCT imaging location	External position markings	Compatible with video bronchoscope instrument channel External position markings every 5 cm
Probe material is safe to biological tissues	Biocompatible materials	PEEK tube Epoxy: Epo-tek 730-110
Prevent perforation/Airway rupture	The probe should have no sharp edges or corners	Distal cap to be made with rounded and soft material
Protection of optical core components during scan	Rigid protection tube surrounding optical components in distal probe	Stainless steel hypotube to protect optics
Isolation of optical core components from patient/organ environment	Watertight static outer housing	Distal end cap, heat shrink tube and epoxy for water proof probe tip

TABLE 3.7: Summary of probe outer sheath mechanical design requirements

unaltered natural mucus flow. In addition, as outer sheath is expected to be in direct contact with the tissues, thorough failure mode analysis and mechanical tests were conducted to ensure subject safety. The sheath design requirements is summarized in Table 3.7.

3.4.2 Fabrication

Polyetheretherketone (PEEK) was chosen for both tubes for its biocompatibility, lateral flexibility, and compressive/tensile stiffness. The outer sheath and driveshaft PEEK tubes were custom-extruded (Zeus, Inc.). To reduce the friction between driveshaft and outer tube during scanning, the driveshaft outer surface was

engineered to have micro-grooves along the tube and Teflon coating was also applied onto the engineered surface. The dimensions of the outer sheath were 1.75 mm inner diameter (ID) and 2.4 mm outer diameter (OD), fitting into the 2.8 mm ID standard bronchoscope instrument channel. The driveshaft was 1.35 mm (OD) and 0.254 mm (ID) to admit a standard optical fiber. In addition, the outer surface of the driveshaft was engineered with a friction-reducing texture and coated with polytetrafluoroethylene (Teflon) to minimize friction during scanning. The outer sheath, which isolated the inner probe from the tissue environment, consisted of an in-house modified PEEK catheter (Zeus. Inc., customized, 1.75mm ID, 2.4mm OD), transparent Iridium polyolefin heat shrink tube (Cobalt Polymers, U2-110-CLR), and an in-house fabricated PEEK cap. The catheter was slotted at the side and machined to reduce its distal diameter by $\sim 150 \mu\text{m}$ to make a step for the transparent heat shrink imaging window (Figure 3.20). The distal end of the disposable sheath was capped with a smooth cover to prevent fluid egress into the probe and tissue injury during insertion (Figure 3.21). A portion of the protective sheath was made of a transparent Iridium polyolefin heat shrink tube to act as an optical window for the μ OCT imaging beam, allowing optical transmission while acting as a moisture barrier.

3.4.3 Performance test

Mechanical tests were performed to ensure the sheath could withstand influence of the forces and environment typical for a clinical, handling procedures and administration to the subjects. The probe should not break along the sheath or at any interfaces during use.

3.4.3.1 Test scheme

The mechanical test scheme for the probe outer sheath is summarized in Table 3.8. These tests were performed to verify the probe robustness.

FIGURE 3.20: Picture of in-house modified PEEK sheath and heat shrink tube as imaging window

FIGURE 3.21: Picture of in-house fabricated PEEK cap under microscope

	TEST NAME	TESTED ITEM	PASS CRITERIA
Mechanical Qualification Tests - Non-Destructive	Watertight	Assembled sheath	No evidence of water intrusion in all materials and interfaces.
	Temperature during prolonged run	Assembled sheath	External temperature less than 43 degrees in all modes of operation
Mechanical Qualification Tests - Destructive	Bend to failure test	Unmodified sheath (PEEK outer tube)	Probe will be rated at a minimum bend radius of 2x the failure bend radius
	Pull Force Test	Unmodified sheath (PEEK outer tube)	No evidence of defects on the interface and materials, especially for signs of breaking or destruction of the sheath at less than 50N
	Transverse Compression	Unmodified sheath (PEEK outer tube)	Sheath will be rated at a maximum force of a half of the failure transverse compression force
	Poking on Imaging Window	Assembled sheath	Imaging Window will be rated at a maximum force of half of the failure poking force
	Axial load	Assembled sheath	Assembled sheath will be rated at a maximum force of half of the failure poking force
	Shear force on entire heat shrink (imaging window)	Assembled sheath wth extended heat shrink tube	Imaging window will be rated at a maximum force of a half of the failure shear force.
	Distal cap dislocation	Assembled Sheath	Distal cap dislocation is rated at the half of the breakage force or maximum available force.
	Fiber breakage	Probe sample	Record fiber breakage force.
	Driveshaft motor dislocation	Probe sample with driveshaft actuator coupler	Record dislocation force

TABLE 3.8: Sheath mechanical qualification test scheme

REQUIREMENTS	INPUTS	POTENTIAL SOLUTION
Work within standard of care of video bronchoscopy	Limited procedure time	< 10 min (SOFT)
Accurate knowledge of μ OCT imaging location	Visual guidance provided	Live continuous video bronchoscopy guidance
Single operator for both bronchoscope and μ OCT probe	Probe should be lockable to bronchoscope	Locking design

TABLE 3.9: Summary of probe ergonomics design requirements

3.5 Probe ergonomics

An ergonomic segment was designed which introduces the probe into the instrument channel of the bronchoscope, fixes its relative position to top of the bronchoscope, contains a means of pushing and pulling the driveshaft through the sheath, and contains connections to the μ OCT console system.

3.5.1 Ergonomics design requirements

Clinical requirements were considered in ergonomics design, such as compatibility of μ OCT endoscopic probe with video bronchoscope instrument channel and accurate knowledge of μ OCT imaging location, etc. as listed in Table 3.9.

While the flexible imaging end of the μ OCT probe is inserted into the bronchoscope for imaging, the proximal end of the probe is held by hand by a trained operator through an ergonomic adapter assembly. The driveshaft is scanned inside the probe sheath by a small motor housed in a water-proof and electrically isolating polymer grip housing. The grip housing holds the disposable outer sheath and reciprocating driveshaft together and can be inserted, retracted, and rotated by hand. When the probe is in position, the grip can be locked in place inside the ergonomic adapter assembly by a simple thumb screw. Figure 3.22 shows the μ OCT probe and probe insertion assembly.

The ergonomic adapter assembly is shown in detail in Figure 3.23. An aluminum insertion guide tube is held in position over the bronchoscope working channel through a lock connector and a support strut, foot and foot strap. A stainless steel

FIGURE 3.22: Bronchoscope with μ OCT probe and probe insertion assembly. a) Bronchoscope distal tip (B), camera port (C) and working channel (W) with the μ OCT probe in the full retracted position. b) Bronchoscope hand control segment with probe insertion assembly installed and in the full retracted position. B: bronchoscope, IT: insertion tube, ICA: Instrument channel adapter, TS: thumb screw, FS: foot strap, MH: motor housing, PT: probe tube, ST: strain relief tube, U: umbilical tube. c) Bronchoscope distal tip with μ OCT probe (P) in the full extension position. T: probe tip, L: laser focus position. Maximum extension is 60 mm. d) Bronchoscope hand control segment with probe insertion assembly in the full extension position.

thumb screw locks the probe insertion tube in place when the probe is in position for imaging.

A small motor is used to scan the driveshaft and it is connected to the fiber-optic components of the μ OCT probe within a flexible outer sheath. The piezoelectric motor is housed inside the water-proof and electrically isolating grip. The motor is connected to a motor controller on the μ OCT console via two cables: a drive signal that provides the electromotive force for the motor, and a position sensor signal that enables closed-loop control of the motor position. These two cables, along with the optical fiber are enclosed in a sealed, flexible umbilical tube which connects to the μ OCT console (Figure 3.24).

3.5.2 Ergonomics fabrication

The handle was fabricated to aid in positioning the probe and to enclose the scanning motor. The handle unit contained the scan-driving motor (Figure 3.25). The

FIGURE 3.23: Ergonomics assembly. ICA: Instrument channels adapter, IT: insertion tube, SA: strut adapter, S: strut, F: foot, FS: foot strap, TS: thumb screw, SS: set screws.

driveshaft was scanned inside the probe sheath by a small linear motor (Physik-Instrumente) housed in a water-proof and electrically isolating polymer grip housing. The grip housing held the outer sheath and reciprocating driveshaft together to allow insertion, retraction, and rotation by hand. A locking thumbscrew allowed fixation of the relative position of the catheter within the instrument channel of the bronchoscope. The flexible imaging end of the μ OCT probe was designed for insertion into the bronchoscope channel for imaging while allowing manual navigation via the ergonomic adapter assembly at the proximal end. The probe was also fully self-contained and was operable independently from the bronchoscope.

FIGURE 3.24: a) μ OCT probe and probe insertion assembly. MH: motor housing, FPS: flexible probe sheath, PT: probe tip, FUT: flexible umbilical tube, CON: connectors to cart system. b) μ OCT probe tip. FPS: flexible probe sheath, HST: heat shrink tubing, TC: tube cap, FOV: field of view. c) probe insertion assembly. MH: motor housing, ST: strain relief tube, U: umbilical tube, PT: probe tube, PSA: probe sheath adapter, FPS: flexible probe sheath. d) U: umbilical tube, OPT: optical connector, MPC: motor power connector, MSC: motor signal connector.

FIGURE 3.25: Bronchoscope with fabricated μ OCT probe insertion assembly. a) Bronchoscope hand control segment with probe insertion assembly installed. b) Fabricated assembly. B: bronchoscope, IT: insertion tube, LA: Lock adapter, TS: thumb screw, FS: foot strap, MH: motor housing, PT: probe tube, ST: strain relief tube. Maximum extension is 70 mm.

	TEST NAME	TESTED ITEM	PASS CRITERIA
Mechanical Qualification Tests - Non-Destructive	Watertight	Probe adapter proximal sub-assembly	No evidence of water intrusion in all materials and interfaces.
	Mechanical Integrity	Probe adapter proximal sub-assembly	Assembled Sheath with Ergonomics is mechanically stable, no loose joints nor moving pieces other than designed to be.
	Mechanical Functions	Probe adapter proximal sub-assembly	Only intended moving parts are mechanically functional. Protrusion of sheath out of the bronchoscope is less than 6cm; sheath tip always stays in the bronchoscope camera view.
	Scan pattern	Assembled probe, sheath, ergonomics	Amplitude of scan $>150\ \mu\text{m}$ at 40 fps. Duty cycle greater than 25%

TABLE 3.10: Ergonomics mechanical qualification test scheme

3.5.3 Performance test

3.5.3.1 Test scheme

The mechanical test scheme for the probe ergonomics is summarized in Table 3.10.

3.5.3.2 Scan pattern

A probe scanning test was conducted with the complete handle assembly to determine the linearity, hysteresis and backlash of the scan. The whole assembly was put through bronchoscope and the bronchoscope was put through a customized airway phantom which resemble human neck bending curvature in upper airway (Figure 3.26).

The driveshaft was connected to a linear piezo actuator (PI, P-602.5SL) at the proximal end via a custom fabricated brass adaptor, and the linear piezo actuator was affixed to the housing by a set screw. A 10V peak-to-peak sinusoidal signal at 40 Hz was used as the command signal for the actuator in closed-loop operation. The unloaded actuator travelled over a range of $\sim 400\ \mu\text{m}$. The beam output at

FIGURE 3.26: Picture of Tearney group airway phantom to mimics human neck bending curvature in upper airway

the distal end of the fully assembled device was traced using a line-scan camera (SPL8192, Basler, Germany) with $10\ \mu\text{m}$ pixel size. Linear regression was conducted for the first rising edge. The coefficient of determination R^2 was calculated for all rising edges in one second.

The scan test demonstrated $\sim 200\ \mu\text{m}$ scanning distance after passing through the full assembly of bronchoscope together with the airway phantom. The mean R^2 was 0.9875 over 40 cycles with standard deviation of 0.0026, which indicates good linearity and repeatability. As the linear motor reversed direction, the driveshaft transitioned between compression and tension, resulting in a constant-position hysteresis period at the crests and troughs of the scan waveform (Figure 3.27b). The duty cycle was approximately 30%.

3.6 *Ex vivo* imaging experiment and discussion

We performed *ex vivo* μ OCT imaging of mouse trachea using both the benchtop μ OCT instrument we utilized in previous studies [42], as well as the flexible probe attached to a typical SD-OCT platform (Figure 3.12). A same-scale side-by-side comparison of benchtop μ OCT images and H&E stained histology section of swine trachea had been published in previous studies [24, 26]. The Figure 3.28 compares two images of excised trachea from mice, one obtained from a benchtop μ OCT

FIGURE 3.27: 10-volt sinusoidal driven signal scanning test for the full ergonomics assembly at 40Hz. a) Beam position recorded by the line-scan camera over time. b) Zoomed in scanning pattern showing two complete cycles to demonstrate repeatability and linearity.

system (top) and the other from the miniaturized flexible μ OCT probe (bottom). In both cases, microanatomical features such as cilia on the apical surface were clearly distinguishable, which indicates that the flexible bronchial probe has comparable imaging performance to that of the benchtop system. This speed and resolution will enable quantitative metrics of functional microanatomy to be determined, such as measures of the thickness of airway surface liquid and periciliary liquid, ciliary beat frequency, and mucociliary transport.

FIGURE 3.28: A comparison of mouse trachea tissue images (*ex vivo*, tissue culture) obtained from a benchtop μ OCT system (top) and from the flexible bronchial probe (bottom). EP: epithelium. Arrows: cilia. Image size: $315\mu\text{m}$ (lateral) $\times$ $243\mu\text{m}$ (axial)

3.7 Laser safety study prior to *in vivo* experiment

To our knowledge, μ OCT has not been attempted in the airway tissue of a human patient *in vivo*. We have endeavored to conduct a study to validate the safety of the μ OCT system, which included the console and airway probe, prior to use in human patients.

3.7.1 Study materials

Swine are an established animal species for pulmonary studies, and the use of swine is accepted for device safety and validation studies by regulatory agencies [55]. The *ex vivo* swine trachea model used in this study is considered equivalent to the *in vivo* situation. This is based on the fact that freshly excised swine trachea (less than three hours postmortem) is still viable and has similar physiological as well as optical properties to *in vivo* human trachea. Three specimens were selected and 7 points were tested on each specimens. The number of specimens obtained from the swine trachea is considered sufficient to provide a meaningful evaluation of test article safety. The swine trachea is obtained from MGH Knight Surgical Animal Laboratory in accordance with the MGH IACCUC protocol 2008N000081. The trachea is harvested from the body with a postmortem interval of less than one-hour and preserved on ice prior to the exposure study. The exposure study is conducted within a three-hour postmortem interval. After all exposures have been completed, the tissue is frozen in preparation for histological staining and assessment.

Light-induced damage is assessed by histochemical staining with nitro blue tetrazolium chloride (NBTC) to positively assess the disruption of the architectural structure of the bronchial airway and loss of cellular viability at the site of the exposure with higher sensitivity and specificity than standard histological stains.

Histochemical staining with NBTC is a well established method used to assess tissue viability in dermatology studies and to visualize myocardial infarction with higher sensitivity and specificity than standard histological stains. The mechanism for assessing viability is based on the reduction of NBTC by a native enzyme,

FIGURE 3.29: Mechanism of NBTC stain for tissue viability. *Adapted from Virchows Arch 393, 287-297 (1981)*

NADH diaphorase [56] (Figure 3.29). NBTC, a redox indicator, undergoes reduction in the presence of an enzyme system with a lower redox potential than the dye itself. In the case of biological systems, the most important redox system is NADH/NAD. When NADH is oxidized in the presence of NADH diaphorase, a direct electron transfer occurs from NADH to the NADH diaphorase enzyme, which in turn, transfers the electron to the NBTC. This reaction reduces NBTC and causes a chemical change from NTBC, a lightly yellow-colored solution, to a water-insoluble blue diformazan precipitate. Electron microscopy indicates that NADH diaphorase is a membrane-bound enzyme located on mitochondria and other cellular structures. If NADH diaphorase is denatured due to thermal damage from laser-induced heating, the reaction cascade is disrupted and the blue diformazan precipitate will not form. Thus, a blue precipitate only occurs in viable cells because diaphorase activity subsides immediately upon cellular death. Using this histochemical method, the border between blue-stained normal and non-stained de-vitalized structures is delineated sharply, permitting accurate determination of the laser-induced damaged zone.

3.7.2 Test system

A system was devised in an *ex vivo* setting to test the architectural disruption and cellular response to the exposure of swine trachea using NKT super continuum excitation light delivered by the MGH μ OCT airway probe. Since the operation of the μ OCT airway probe will follow the operational procedures established for the

FIGURE 3.30: Experimental schematic used for validating the safety of μ OCT exposure parameters.

clinical μ OCT airway imaging protocols, parameters are chosen to reflect similar conditions with increased exposure power and time of exposure.

The experimental setup of the test system is shown in Figure 3.30. The light source used in the μ OCT console is based on a super continuum fiber laser (NKT Photonics; SuperK Extreme), which produces a maximum power of 5.8 W over a wavelength range of 400 to 2400 nm. The usable part of the μ OCT spectral band is 650-1000 nm. Light from the laser is collimated and passes through a 90:10 beam splitter with 10% of the power is coupled into the MGH μ OCT probe. The output energy of the fiber is measured using a calibrated power meter. For this safety study, no spectral filtering occurred prior to coupling the light into the μ OCT probe, therefore all wavelengths, including NIR wavelengths, are included in the output. This represents the worst-case exposure scenario.

The distal end of the μ OCT airway probe is terminated with a GRIN lens that focuses the light onto the tissue surface. The probe is placed in near contact with the luminal trachea surface, until the smallest focused light excitation spot ($2.2 \mu\text{m}$) is produced on the trachea surface. The tissue is immersed in PBS solution maintained at 37°C . A two-dimensional translation stage advances the probe from

FIGURE 3.31: Schematic illustrating the pattern of exposure sites with positive registration controls. Sites 1 and 7 are labeled with ink and act as both positive controls for tissue damage and visible registration sites. Sites 2-6 are exposed for a duration of 15, 30, 50, 120 and 180 seconds respectively at 42 mW.

one exposure site to the next and an electronically-controlled shutter in the beam path determines the exposure time. There is no light exposure when the probe is positioned between exposure sites.

Rationales of experimental variables tested during the trachea *ex vivo* study are:

1. Optical power: In benchtop mice trachea studies, ~ 15 mW optical power is delivered onto sample to visualize ASL, PCL, CBF and MCT. The sensitivity is above 85 dB. It is expected that clinical study will use similar optical power to achieve a sensitivity of at least 80 dB, with maximum power achievable of 20 mW. Safety validation study will increase power to 42 mW, 2x greater optical power on tissue than the maximum possible optical power that can be encountered using the MGH μ OCT airway probe.
2. Exposure time: Clinical study has an exposure limit of 30 seconds controlled by mechanical shutter. Safety validation study will increase the exposure time to 180 seconds, 6x greater exposure duration on tissue than the maximum possible exposure duration that can be encountered using the MGH μ OCT airway probe.

3.7.3 Experimental procedures

Whole-thickness trachea sections ($n=3$) dissected from freshly excised swine trachea are exposed to light delivered by the μ OCT probe. Each tissue specimen has five exposure sites in addition to two positive control sites. For each test site, a

single light exposure is delivered by the μ OCT probe for a specified duration at an exposure power of 42 mW. Positive damage controls are generated with the aid of tissue marking ink, which strongly absorbs the light and subsequently heats the tissue producing damage. Areas that have not been exposed to light act as negative controls for damage and act as check upon the quality of the NBTC stain. After each exposure study, the tissue was frozen, histologically thin-sectioned and stained with NBTC to evaluate architectural damage and cellular viability.

An important consideration in the experimental design is establishing the accurate registration of the exposure sites. For each tissue section, a total of five sites were exposed to 42 mW for 15, 30, 60, 120, 180 seconds delivered by the μ OCT probe, as shown in Figure 3.31. Each tissue exposure is performed under a non-flowing PBS solution with a non-rotating probe. The minimum spot size is 2.2 μ m in diameter (FWHM). After all exposure sites are illuminated, two registration marks located at sites 1 and 7 are burned into the tissue with the aid of red and green colloidal ink using longer exposure times (> 180 seconds). These registration sites are illuminated until visible damage is observed on the luminal surface. The registration sites served to preserve the tissue orientation during histological cutting and to act as a positive control for the NBTC stain.

After the tissue is exposed and the registration markers generated, the tissue is frozen until histological processing. Prior to sectioning, the tissue is trimmed close to the registration ink marks and mounted in OCT (Optimal Cutting Temperature) media. Because the NBTC stain must be performed on viable, unfixed tissue, all sectioning is done on fresh-frozen blocks. Multiple histology levels are cut starting at the edge of the ink and sectioned through the spot at 100 μ m levels. The sections are stained with NBTC using well-established methods.

3.7.4 Experimental results

Representative NBTC-stained sections for this exposure study are shown in Figure 3.32. The sections are uniformly stained blue in the cellular regions indicating good NBTC staining. The appearance of unstained tissue under the ink on the luminal surface indicates the location of both registration sites. Since the tissue exposure sites (2-6) are arranged linearly between the registration sites, the exposure sites

FIGURE 3.32: NBTC-stained histology slides from swine trachea A, swine trachea B and swine trachea C. The sections are cut to a thickness of $10\ \mu\text{m}$ to enhance the sensitivity of the stain. Scale bar: 1mm.

are histologically captured by cutting through the registration ink spot with a high degree of confidence.

In Figure 3.32, no tissue damage is observed at the five (5) controlled exposure sites on each of the sections. The trachea wall appears normal with no evidence of architectural disruption. There is a strong, uniform blue NBTC stain throughout tracheal surface indicating viable tissue with no disruption of the architecture. The collagen rings within the trachea stain yellow as anticipated.

When visible, the epithelial cells also stained blue. Positive control sites are indicated and show the expected clear unstained regions surrounded by blue-stained viable tissue.

Histology views at higher magnification are shown in Figure 3.33. When visible, the epithelial cells also stained blue. Positive control sites are indicated and show the expected clear unstained regions surrounded by blue-stained viable tissue (Figure 3.33D). A summary of the results is provided in Table 3.11. For the specimens investigated, no tissue damage was identified at 42 mW continuous exposure for up to 180s.

FIGURE 3.33: High-resolution NBTC histology images (6.3x) from trachea. (A-C) A dark blue stain is observed at tissue and indicates tissue viability after the laser exposure at site 6 of sample A, B and C respectively. (D) At tissue site 1 of sample A (positive control, 42 mW at >180 s), green ink is seen on the luminal surface and a lack of dark blue stain indicates that the tissue is no longer viable. Scale bar (100 μ m) is shown.

	1	2	3	4	5	6	7
Power (mW)	42	42	42	42	42	42	42
Time (s)	> 180	15	30	60	120	180	> 180
Trachea 1	+	-	-	-	-	-	+
Trachea 2	+	-	-	-	-	-	+
Trachea 3	+	-	-	-	-	-	+

TABLE 3.11: μ OCT exposure results. (+) indicates that damage was observed as evidenced by loss of blue stain. (-) indicates that damage was not observed as evidenced by no loss of blue stain. Positive Control: where absorbing ink was placed on the specimen.

FIGURE 3.34: μ OCT imaging console showing sub-systems overview FIGURE 3.35: μ OCT imaging console showing front view

3.8 *In vivo* swine study

3.8.1 Study setup

Figure 3.34 and Figure 3.35 are schematic drawings of the μ OCT imaging console design and they illustrate how the μ OCT console interfaces with the μ OCT endobronchial probe. During *in vivo* imaging, a system operator controlled the

console while a clinician operated the bronchoscopy probe. The system operator was responsible for the operation of the laser, motor controller, synchronization electronics and image acquisition software, while the clinician was responsible for positioning the bronchoscopy probe in the patient. The main functions of the console were to provide a laser source for the bronchoscopy probe, to control the piezoelectric motor that drives the probe optics, to collect reflected laser light from the probe and acquire spectrograms, to stream spectrogram data to disc, and to render and display live OCT images on the display. After imaging was completed, the console transferred the *in vivo* data securely to clinical data management computers for archival and interpretation.

The optical subsystem included the following critical components :

1. Filter set.

The filter set comprised a custom-made dichroic beam splitter and a spectral shaping filter. This set of optical components attenuated and reflected the incident beam in a manner that ensured most of the light delivered downstream was within the 580-1000 nm bandwidth and possessed an approximately Gaussian spectral distribution. The reflected beam carried a maximum of 1.15 W of total optical power at 100% laser power. The unwanted reflected optical power was directed towards a beam block.

2. 70:30 beam splitter.

The spectrally filtered beam entering the beam splitter was split into two arms. 30% was transmitted and 70% was reflected. Furthermore, an optical power meter monitored the spectrally filtered light. The transmitted beam was directed into an achromatic objective lens that coupled the light into the probe. As an additional safety feature, a mechanical shutter was set between the beam splitter and the objective lens. The returning beam from the probe entered the beam splitter and 70% of the light was directed towards the spectrometer.

3. 940 lp/mm volume phase holographic grating.

The grating spectrally dispersed the returning interferogram from the probe.

4. Nikon camera lens attached to a Basler 4096-pixel line scan camera.

FIGURE 3.36: μ OCT imaging console optics sub-systems design overview FIGURE 3.37: Picture of μ OCT imaging console optics sub-systems

The lens imaged the spectrally dispersed interferogram onto the line scan camera, where the signal was digitized and sent to a frame grabber in the camera through a master and slave camera link cables. A 20.48 kHz digital TTL signal from the digital output channel of the function generator controlled the camera exposure and triggers frame grabber data acquisition.

All the optical components described in this section were mounted on an optical breadboard attached to a sliding drawer in the enclosure rack as shown in Figure 3.36 and Figure 3.37.

3.8.2 Study procedures

Two ~ 50 kg swine were prepared for bronchoscopy. They were sedated, intubated with 7.5 ETT and anesthetized with isoflurane and telazol (ketamine + xylazine), with no atropine. Position was supine. In some cases, especially after imaging the lower airways, the bronchoscopy procedures were halted to allow the animal to recover from low O_2 sat (SpO_2) because of typical airway obstruction from bronchoscopy. The swine had a mean heart rate of approximately 100 bpm and a respiratory rate of 9-16 times per minute. There were sporadic areas of mucus, similar to other normal swine.

During the procedures, the bronchoscope was inserted into the trachea and a region of interest was identified, starting with the proximal trachea. Mucus was suctioned to clear the airway as needed. The μ OCT probe was then threaded through the instrument channel of the bronchoscope.

As the probe passed the distal tip, the probe could be viewed under bronchoscope in the airway. The μ OCT probe was rotated to position the window against the airway wall. The laser was enabled, foot pedal pressed and light could be seen by the bronchoscope. At this point, the operator had to view both bronchoscopy and μ OCT screens to optimize the position of the probe for the best images. Rotation of the inner cannula of the μ OCT probe was frequently needed to align laser output with imaging window. When the bronchoscope was retracted from the airway because of patient (animal) concerns, the μ OCT probe was retracted from the bronchoscope first.

Three investigators were needed in the study: clinician to maneuver the bronchoscope; assistant to maneuver the μ OCT in the scope; and μ OCT console operator.

3.8.3 Study results

The first *in vivo* experiment with this flexible endobronchial probe showed promising results in terms of optical performance. We were able to visualize beating cilia, PCL, ASL, epithelium, mucus, and blood flow during in a swine model, as shown in figure 3.38 and 3.39. We measured PCL to be $\sim 7 \mu\text{m}$. ASL varied from $20 \mu\text{m}$ to a few tens of microns. Debris were trapped by mucus along airway surface.

FIGURE 3.38: *In vivo* experiment images with flexible endobronchial μ OCT probe in swine airways showing ASL and PCL.

In addition, we also observed a blood vessel with red blood cells flowing inside, demonstrated in Figure 3.39b.

The biggest issue limiting quantitative analysis of the dynamic MCT functions was the significant motion artifacts introduced by probe positioning, heartbeat and breath. Although cilia's beating was visually observed, its frequency was not reliable. Since both the field of view and depth of field of the probe were less than $400\mu\text{m}$, positioning the probe to the right regions of interest was very difficult,

FIGURE 3.39: *In vivo* experiment images with flexible endobronchial μ OCT probe in swine airways showing PCL, Ep and blood vessel

especially for the first animal when physicians were not familiar with the operation procedures. Even after finding the right position, maintaining probe relative stable to the tissue was also difficult. In addition, breath artifacts caused the targeted tissue to move in and out of the field of view, resulting in periodic loss of view. It was mitigated by making the animal to hold its breath temporarily. Heartbeat artifacts were observed as the tissue moving up and down in synchronization with the heartbeat monitor beeps. Better mechanism to fix the probe relative position to the tissue without direct contact to disturb airway physiology functions is desired.

3.9 Summary and outlook

In this chapter, we presented an improved and clinical translatable endobronchial μ OCT probe for visualization of MCT in lower airways.

The 2.4 mm diameter and flexibility of the probe allows it to be inserted into the instrument channel of a standard bronchoscope, enabling real-time video guidance of probe placement. Each probe consists of three main parts:

1. Inner core

It transmits optical signals to and from the endobronchial probe. It includes optical components, a protection hypotube and a driveshaft.

2. Outer sheath:

It isolates inner core from the tissue environment and protect it from other damages during normal procedures. It includes an over tube, a distal cap and an imaging window

3. Ergonomics:

It introduces the probe into the working channel of the bronchoscope, fixes its relative position to top of the bronchoscope. It includes a motor enclosure, a scanning device that pushes and pulls the driveshaft and an in-house fabricated grip that can be attached and locked onto bronchoscope instrument channel.

Each segment was tested individually to make sure all design requirements were met for clinical application. A laser safety study was conducted before *in vivo* experiment to confirm the tissue safety under broadband, high power laser illumination.

In vivo experiment with swine showed anatomical structure information of animal airways but quantitative analysis was limited by significant motion artifacts and the unknown exact scan range information.

Nevertheless, this flexible endobronchial μ OCT probe was the first step towards clinical μ OCT application and opens possibility to image cilia and mucociliary transport in small distal airways *in vivo*, where the pathogenesis of lung diseases such as cystic fibrosis (CF) and chronic obstructive pulmonary disease are believed to originate, allowing the underlying mechanisms of these pathologies to be studied. Further, patient-specific imaging of mucociliary function would allow personalized monitoring and tailoring of interventional treatments, which would be particularly

valuable in diseases such as CF that frequently require cocktail pharmacologic therapies.

Chapter 4

Ultrahigh resolution dual spectrometer μ OCT

The chapter describes a μ OCT imaging system that achieves an axial resolution of $\sim 1.27 \mu\text{m}$ in air which, to the best of our knowledge, is the highest axial resolution OCT using NIR SLD sources up to date. Section 4.1 introduces previous studies for high-resolution OCT. Section 4.2 and Section 4.3 describe the experiment system setup and characteristics, including resolution, chromatic focal shift, phase stability and sensitivity. Imaging was conducted on zebra fish larvae to visualize cellular details, which is discussed in Section 4.4. Section 4.5 summarizes this chapter.

4.1 Background and literature review

Over the past 20 years, research has been focused on high resolution technologies with resolution from tens of micrometers to one micrometer in an attempt to achieve cellular and subcellular level imaging [26, 27, 57–60]. It has been demonstrated that many cellular and sub-cellular structures of biological tissues can be reliably visualized using a class of imaging techniques characterized by $\sim 1\text{-}\mu\text{m}$ axial resolution [23, 24, 27, 58, 60]. For example, it has been recently revealed that many cellular and subcellular structures and processes associated with coronary artery disease can be clearly visualized using 1-micron resolution OCT [27], which has never been reported before. Another example is evaluation of mucociliary clearance process in association with Cystic Fibrosis airway disease, which would

require the use of novel 1-micrometer resolution OCT systems in order to spatially resolve them [23, 24, 26, 61, 62]. In order to achieve such a high axial resolution, repetitive pulsed laser sources have been used to obtain a large spectral bandwidth and visible light was included at the blue end of the spectrum in favor of the improved resolution [26, 27, 58, 60, 63]. As near infrared (NIR) radiation from superluminescent diodes (SLD) is less likely to cause tissue damage than visible, repetitive pulsed radiation, a 1- μm axial resolution OCT technology which operates with NIR, SLD light sources is desirable especially for ocular and skin imaging applications *in vivo*.

In this chapter, we report a μOCT imaging system that achieves an axial resolution of $\sim 1.27\ \mu\text{m}$ in air using NIR SLD sources and dual spectrometer design. The benefits of using a dual spectrometer design are 1) possibility to improve axial resolution without safety concerns; 2) possibility to improve penetration depth without compromising axial resolution. For a single spectrometer based OCT system to achieve axial resolution better than $1\ \mu\text{m}$ in tissue, the practical option is to shift the spectrum to the blue end which will unavoidably decrease the penetration depth and includes visible radiation where much stricter safety regulations than NIR radiation apply for *in vivo* applications. The two-spectrometer design makes it possible to improve axial resolution using NIR radiation and gain improved penetration by use of longer center wavelength.

4.2 System overview

The imaging system presented uses two SLD arrays to provide NIR illumination from 755–1105 nm (3-dB spectral range) (Figure 4.1). In order to detect spectral interference signal across the entire illumination bandwidth, we employed two spectrometers based on an InGaAs camera (Sensors Unlimited GL2048L) and a Si camera (E2V, AViiVA EM4) respectively. There is a $\sim 100\text{-nm}$ overlap, which allows us to combine spectral interference signals acquired from the two cameras with high accuracy in spite of differences in optical arrangements between the two spectrometers (Figure 4.2 (a)).

The construction of the imaging system is depicted in Fig. 1. The SLD array 1 (Superlum Broadlighters T-850-HP) provides optical power of 19.3 mW over a

FIGURE 4.1: One-micrometer axial resolution optical coherence tomography system. L1-8: lenses. M1-3: mirrors. BS1-3: non-polarizing cube beam splitters. SMF: single mode fiber. RM: reference mirror. NDF: neutral density filter. G1-2: gratings. PC: personal computer. GS: galvo scanners.

3-dB spectral range of 755-930 nm, and the SLD array 2 (Exalos Ultra-Broadband EBS4C32) offers optical power of 5.72 mW over a 3-dB spectral range of 938-1105 nm. The output of two SLD arrays is combined using a 70:30 (R:T) non-polarizing cube beam splitter (BS1) to balance the spectral power density at the output of the beam splitter. The combined optical beam is guided to the desktop probe through a 50:50 non-polarizing cube beam splitter (BS2) and a single mode fiber (Nufern, 780-HP). In the desktop probe, the light beam is collimated by an achromat (L8, AC050-015-B, Thorlabs Inc.) and then split into two wavefronts by a 45° rod mirror (NT47-628, Edmund Optics Inc.) The central circular wavefront is directed to the reference arm and the annular wavefront goes to the sample arm. The optical power on the sample is 690 μ W. An objective lens (Mitutoyo Plan Apo NIR, 20X, NA = 0.4, $\sim$ 70% transmission) is used to focus the light beam into a focal spot of 1.7 μ m (full-width at half maximum, FWHM). Light backreflected from the reference arm and backscattered from the sample arm is recombined through the rod mirror and guided by the single mode fiber back to a 10 μ m pinhole via the 50:50 beam splitter (BS2). The interference signal is split by another 50:50 non-polarizing beam splitter (BS3) to the two spectrometers. The spectrometer 1 is composed of an 830 lines/mm ruled grating (NT43-850, Edmund Optics Inc.), a camera lens (Nikon AF Nikkor 85mm f/1.8D), and the Si based linear sensor. We use 1050 camera pixels to detect a total spectral range of 850 ± 100 nm. The spectrometer 2 is composed of a 1,200-lines/mm transmission grating (830 nm, Wasatch Photonics Inc.), a camera lens (Nikon AF Nikkor 50mm f/1.8D), and the InGaAs based linear sensor. We use 1,350 camera pixels to detect a total spectral range of $1,020 \pm 80$

nm. For each linear sensor, we digitized the detected signals at 12-bit resolution and transferred them to the personal computer through camera link cables and an image acquisition board (KBN-PCE-CL4-F, Bitflow). Both cameras and the galvo scanner are synchronized by a triggering signal generated by the computer.

4.3 System characterization

4.3.1 Spectrometer calibration

We developed a semi-automatic spectral mapping algorithm. Firstly, a set of calibration interference fringes was acquired from both cameras when a partial reflector is located at the focal plane of the sample arm focusing optics. By changing the reference delay, this step was repeated at two optical pathlength differences between the two arms. After background subtraction, we secondly obtained the indices of the zero crossing points of the calibration spectra using linear interpolation. In the third step, we manually selected a subset of a spectrum in the overlap region acquired from the Si camera which started from the n th ($n = 1, 2, \dots, N$, N is the total number of zero crossing points) zero crossing point and ended with the $n+9$ th zero crossing point. We then conducted an exhaustive search for the best match between the selected spectral subset and subsets of 10 consecutive zero crossing points in the corresponding spectrum from the InGaAs camera acquired at the same optical pathlength difference. Finally, index mapping data that provided the best match was verified with the other two sets of calibration interference fringes acquired from the other two pathlength differences. By compounding two spectrometers using the index mapping data, an extended FWHM spectral range of 293 nm centered at 927.5 nm was achieved (Figure 4.2 (b)).

4.3.2 Resolution

The axial resolution resulted from the combined spectrum was tested to be $\sim 1.27 \mu\text{m}$ in air which corresponds to $0.93 \mu\text{m}$ in tissue ($n = 1.36$) (Figure 4.3 (a)), red dashed profile), which matches well with a theoretical resolution of $1.16 \mu\text{m}$ in air. The axial sidelobes of -5 dB are due to spectral fluctuations of both SLD arrays and the decayed spectral responses from 850 – 980 nm of both cameras. A spectral

FIGURE 4.2: Spectral compounding. Interference spectra acquired using a reflective interface by (a) the Si camera (green) and the InGaAs camera (cyan). (b) Combined interference spectrum demonstrating an extended spectral range. Red curves: envelopes of spectrometer output.

shaping method was implemented to reduce the spectral intensity fluctuation [64]. In Figure 4.2 (a) and Figure 4.2 (b), the red line outlines the envelope of original signal and the blue curve is the compounded spectrum after sidelobe suppression.

The improved axial profile (purple solid line in Figure 4.3 (a)) shows that the sidelobes are suppressed by ~ 2 dB without significant degradation of axial resolution. We also measured axial point-spread functions (PSF) at various depths of a mirror sample by changing the delay in the reference arm. The results show that the axial resolution decreased to $\sim 1.7 \mu\text{m}$ at a depth of 0.5 mm where signal dropped by 6 dB (Figure 4.3 (b)). To test the system stability, we monitored the axial profile over 10,000 sec and observed little change in the axial resolution.

FIGURE 4.3: (a) Measured axial point-spread function using an attenuated mirror; (b) Measured axial PSFs on a linear scale for different delays relative to the reference arm length; (c) Axial resolution as a function of distance to focal plane

4.3.3 Chromatic focal shift

Chromatic focal shift is an important factor to cause axial resolution variation along the depth direction in high resolution OCT systems. To characterize the dependence of axial resolution on the chromatic focal shift of the sample arm optics (L6), we simulated spectral distribution at each depth along a focal range of 80 μm using Zemax (Zemax LLC, Redmond, WA), assuming the apochromatic objective lens is perfectly corrected for chromatic focal shift in the working wavelength range. Theoretical axial resolution was estimated based on the simulated center wavelength and spectral bandwidth (Figure 4.3 (c), blue stars). The measured values (Figure 4.3 (c), red circles) match well with the simulation results.

4.3.4 Phase stability

In order to test the phase stability of this dual-spectrometer scheme with respect to a single spectrometer system, we measured phase variations of all the pixels over 512 A-lines with an optical pathlength difference of 0.09 mm. The line rate and the exposure time of both cameras were 10,000 Hz and 90 μs respectively. The phase stability is defined as the standard deviation of the phase differences between sequential A-lines according to reference [65]. The results demonstrate

FIGURE 4.4: Standard deviation of the phase difference between sequential A-line as a function of wavelength for spectral compounding system

that the mean phase stability within the overlap region is around 9% higher than that outside of the overlap region (Figure 4.4), which may be partially attributed to the spectral intensity variation.

4.3.5 Sensitivity

To characterize the sensitivity of the system, we measured the signal to noise ratio (SNR) using a partially-reflecting mirror sample (-40.7 dB reflectivity) when the pathlength difference between the mirror sample and reference arms was maintained at 0.1 mm. The sensitivity for the system (combined spectrum), Si camera alone, and InGaAs camera alone was measured to be 95.8 dB, 95.4 dB, and 91.1dB respectively.

In order to understand the measured sensitivity, we analyzed the SNR in the case of individual camera using the following formulas [66]:

$$S[\text{dB}] = 10 \times \log_{10} \left(\frac{N_{ref} \times \sum N_S}{N_{sh}^2 + N_{el}^2 + N_{RIN}^2} \right) \quad (4.1)$$

$$N_{RIN} = \left(\frac{f}{\Delta v} \right)^{\frac{1}{2}} N_{ref} \quad (4.2)$$

Spectrometer number	1 (Si)	2 (InGaAs)
$N_{el} (e^-)$	846	872
$N_{RIN} (e^-)$	72	56
$N_{sh} (e^-)$	503	398
Δv (GHz)	68.82	44.4
Spectrometer efficiency	0.31	0.40
Full well depth (e^-)	312,500	2,100,000
$N_{ref} (e^-)$	252,670	158,320
$\sum N_S (e^-)$	2,291,100	1,100,200

TABLE 4.1: Parameters for SNR analysis

$$N_{sh} = N_{RIN}^{\frac{1}{2}} \quad (4.3)$$

where N_{ref} is the number of electrons per pixel generated by the reference arm light, N_{el} is the electrical noise of the photo detector, N_{sh} is the number of shot noise electrons, f is the reciprocal of twice the exposure time of the CCD, Δv demotes the FWHM spectral bandwidth of the reference light received by single pixel, N_s denotes the sum of electrons over the entire array generated by sample arm light returning from a 100% reflector. Since the two cameras are synchronized, the line rate and the exposure time of both cameras were 10, 000 Hz and 90 μ s respectively. The parameters mentioned in Equation 4.1, 4.2 and 4.3 for SNR analysis are listed in Table 4.1 for the spectrometer 1 and 2 respectively. The amount of noise generated per camera pixel was expressed in terms of the number of noise-equivalent electrons accumulated in each camera exposure (A-line) [66]. For the Si camera, the total electrical noise was found to be 846 e^- . The relative intensity noise (RIN) was calculated to be 72 e^- with the number of electrons per pixel generated by the reference arm light of 252,670 e^- . At 10kHz scan speed, the Si camera operated at an exposure time around 90 μ s. The FWHM spectral bandwidth of the reference light received per pixel was calculated to be 0.164 nm. With a mean spectrometer efficiency of 0.31 (including the grating diffraction efficiency, quantum efficiency of the sensor, and camera lens efficiency), a maximum SNR per pixel could be reached if the number of electrons per pixel generated by the reference arm light was equal to 2,968,873 e^- which is 9.52 times larger than the full well depth (312,000 500 e^-). The number of shot noise electrons was calculated to be 503 e^- . Therefore, the total noise power of our system with the Si camera alone was 2.83 times larger than the shot-noise limit. The sum of electrons over

the entire array generated by sample arm light returning from a 100% reflector was 2,291,100 e^- . Therefore, assuming a uniform spectral density, the sensitivity was predicted to be 98 dB. We think the 3-dB difference between the measured sensitivity and the theoretical prediction is mainly due to non-uniformity of the spectral response of the camera.

In the case of InGaAs camera, the total electrical noise was found to be 872 e^- . The RIN was calculated to be 56 e^- with the number of electrons per pixel generated by the reference arm light of 158,320 e^- . Since the two cameras are synchronized, the line rate and the exposure time of the InGaAs camera was also 10k Hz and 90 μ s respectively. The FWHM spectral bandwidth of the reference light received per pixel was calculated to be 0.148 nm. With a mean spectrometer efficiency of 0.40, a maximum SNR per pixel could be reached if the number of electrons per pixel generated by the reference arm light was equal to 2,465,269 e^- which corresponds to 117% of the full well depth (2,100,000 e^-). The number of shot noise electrons was calculated to be 398 e^- , and the total noise power of our system with the InGaAs camera alone was 3.33 times larger than the shot-noise limit. The sum of electrons over the entire sensor generated by sample arm light returning from a 100% reflector was 1,100,200 e^- . As a result, the sensitivity was predicted to be 93.46 dB. We attribute the difference between the measured sensitivity and the theoretical prediction to non-uniformity of the spectral response of the camera over the spectral range from 930 – 980 nm. Assuming a uniform spectral density, the sensitivity was predicted to be 98.44 dB and 93.46 dB for the spectrometers 1 and 2 respectively.

It is expected that the measured sensitivity of combined spectrum is higher than the measured sensitivity of individual camera. This is because the two spectra have a $\sim$ 100-nm overlap where the noise was suppressed relative to the signal based on the SNR model [66]. Because the full well depth of the Si camera is 6.72 times smaller than that of the InGaAs camera, under the condition of a uniform input spectral density and a uniform spectral reflectivity of the reference arm, each InGaAs camera pixel was only 7.54% filled when the Si camera was saturated. The InGaAs camera should have had $\sim$ 5.5 dB higher sensitivity given enough reference light power in the range of 930-1105 nm. This problem can be solved by using a light source with higher power or more efficient system design. In addition, the

sensitivity can be further improved by increasing the sample arm light power at the cameras using an objective lens with higher transmittance rate.

4.4 Imaging experiment and discussion

In order to validate the proposed system for *in vivo* cellular resolution applications, imaging was conducted using three-day-old zebra fish larvae from the dorsal side near the tail. Images were recorded over a field view of $873\ \mu\text{m} \times 436\ \mu\text{m}$ (transverse $\times$ longitudinal) at a frame rate of 20 Hz with 512 lines per frame. Numeric dispersion compensation was used to balance the water dispersion. In a representative image (Figure 4.5 (a)), the blood flow (Green box and the inset) in the artery can be clearly visualized near the notochord. We identified a few similar reflective/scattering patterns in each frame, and each pattern was composed of two bright and distinct interfaces with $\sim 3.3\ \mu\text{m}$ ($n = 1.36$) spacing in between (Figure 4.5 (a-d), orange arrows). Based on the width and height of individual signal pair, we think that each of them represents the top and bottom surfaces of individual disk-like [67] red blood cell as illustrated in Figure 4.5 (e). We further identified flat, spindle shaped structures lining along the luminal surface of the blood vessel wall (Figure 4.5 (a-d, f), green arrows). Based on the shape, dimension, and anatomical location (Figure 4.5 (e)), these structures are possibly endothelial cells.

4.5 Summary and outlook

A dual spectrometer design is more powerful than the single spectrometer design in high resolution applications. For a single spectrometer based OCT system to achieve axial resolution better than $1\ \mu\text{m}$ in tissue, the practical option is to shift the spectrum to the blue end which will unavoidably decrease the penetration depth and include visible radiation where much stricter safety regulations than NIR radiation apply for *in vivo* applications. The dual spectrometer design makes it possible to improve axial resolution using NIR radiation and gain improved penetration by use of longer center wavelength.

In conclusion, we developed a one-micrometer axial resolution OCT system using NIR SLD light sources, which gives the highest resolving power comparing

FIGURE 4.5: *In vivo* one-micrometer resolution images of zebra fish larvae. (a) A representative imaging showing the tail artery (green box and inset) near the notochord (N). Scale bars in the main figure: $50\ \mu\text{m}$; Scale bars of inset: $25\ \mu\text{m}$; (b)-(d) Each RBC appears as individual signal pair (orange arrows) originating from the top and bottom surfaces of the disk-like cell body. (e) Illustration of endothelium and RBCs in an artery. (f) Structures at the surface of the vessel wall suggest endothelial cells (Green arrows) in a 10-frame averaged image. Scale bars: $25\ \mu\text{m}$.

to other systems with same classes of light sources. We have validated the axial resolving power of the proposed system in zebra fish larvae by visualizing cellular structures *in vivo*. Our system employs NIR radiation from SLD sources which is safer compared with the repetitive-pulsed source with visible radiation [26, 27, 58, 60, 63, 68]. In addition, the proposed imaging system is also superior in cost and sensitivity compared with existing high resolution system employing high brightness broadband light sources [26, 27, 63, 68]. Based on these facts, the proposed system provides a viable solution for one-micrometer resolution cellular level OCT imaging *in vivo*. Future researches could be conducted to integrate this dual-spectrometer design with the μ OCT endoscopic probe presented in chapter 3 for a safer and higher-resolution clinical diagnostic imaging tool.

Chapter 5

Multifiber μ OCT probe for speckle reduction

This chapter presents a MFOCT system for speckle reduction in spectral domain OCT. The speckle-reduced images exhibited better image contrast, SNR, CNR and ENL compared to the original images without loss of resolution. Section 5.1 gives a background introduction on the importance of speckle reduction in high resolution OCT and overview of previous studies for speckle reduction techniques in OCT. Section 5.2 and Section 5.3 describes the experiment system setup and characteristics. Imaging was conducted on rat esophagus to demonstrate a better delineation of layered tissue microstructures, which is discussed in Section 5.4. Section 5.5 summarizes this chapter.

5.1 Background and literature review

Although spatial resolution of μ OCT (1-2 μ m) is theoretically high enough to resolve epithelial cells, in most cases few cellular structures can be detected [23, 24, 26] because speckle reduces imaging contrast.

Speckle is the granular pattern observed in the coherent detection of scattering region, appearing when waves of random phases interference with each other. It greatly reduces the contrast between small intrinsic features with subtle differences and significantly limits the resolving power of current μ OCT systems. It is visible

FIGURE 5.1: Comparison of OCT images with and without speckle reduction technique. (a) Original OCT image. (b) Speckle reduced OCT image by taking average of 57 images with visible cell nuclei

when signal is backscattered from a rough surface and is found in all types of coherent detection systems such as radar, ultrasound, radio astronomy, and optical coherence tomography [69]. Sample properties such as morphology structures and motions can randomize the phase of light backscattered from the sample and cause speckle. Properties of light sources and sample beam optics can also contribute to the generation of speckle.

Unlike noise, speckle is usually static in time since it arises from the interference of distorted wavefronts backscattered from uneven biological tissues and backreflected from the reference beam [69]. Different portions of light experience random path length delay when they propagate through the biological tissue and are backscattered by random distributed particles with arbitrary angles. Light becomes random in phase and the combined wavefront is corrupted. When distorted wavefront interferes with the reference beam, it produces visible speckle in obtained images.

In the case of μ OCT, speckle limits the resolving power of μ OCT. Due to the merit of high resolution, μ OCT is able to resolve microbiological features to the size of 1-2 μm , which is comparable to the size of speckles. Thus, it is more difficult to differentiate small tissue intrinsic features from random speckles. Such as in Figure 5.1, OCT image after speckle suppression (Figure 5.1 (b)) gives visible cell nucleus whereas this important feature is hidden behind speckle noise in Figure 5.1 (a). Figure 5.1 demonstrates that a simple speckle suppression technique such as inter-frame averaging can reveal new information critical for disease diagnosis. Therefore, there is a valid reason to believe that more biological features could be revealed with more effective speckle reduction techniques. Most of the time, the differences

between normal and abnormal tissues are small and extremely hard to be detected, but they are critical in disease diagnosis since the subtle differences indicate the transition of tissues from health to disease. Therefore, speckle reduction is of significant importance in disease diagnosis such as early cancer detection. It is desired that we are able to distinguish unhealthy from healthy and make a definite diagnosis as early as possible to start the intervention in clinical cases.

Speckle suppression techniques have been proposed and explored for better OCT image quality by compounding uncorrelated speckle patterns acquired at different polarizations, locations, times or frequencies.

Implementing polarization diversity to reduce speckle can be automatically realized by most OCT scanners with non-polarization-preserving single-mode fibers based interferometers. The polarization-independent fiber-optics reflectometry demonstrated in [70] is believed to work equally well in OCT. A limitation of polarization diversity is that SNR can only be improved by a factor of $\sqrt{2}$ at most since there are only two orthogonal polarization states.

Frequency compounding for speckle suppression was developed and demonstrated by Pircher et al. on fingertip *in vivo* [71]. Two different center frequencies of non-overlapping emission bands, 1312nm and 1488nm, were used as the light sources. An increase in the image quality and improvement in SNR by a factor of ~ 1.56 were observed without degradation in spatial resolution. However, the system complexity was increased and would be more complex for further enhancement in SNR. More importantly, the number of uncorrelated speckle patterns was achieved at the cost of axial resolution because of the splitting of bandwidth in frequency compounding.

Angular compounding has proven to be immune to such drawbacks and showed improved delineation of tissue microstructure. Angular compounding can suppress speckle noise by a factor of $\sqrt{N}$, where N is the number of angles along which speckle is not correlated from each other, without significant degradation in resolution. Considering the merits of angular compounding over the above mentioned techniques, research interest has been focused on angular compounding for speckle suppression.

Angles can be varied sequentially using a rotational/translational mirror. The OCT system demonstrated in [72] can acquire 400 angularly resolved images with

backscattering angular range of 32 degrees. As high as 8dB SNR improvement was achieved. Similarly in [73], the backscattering angular range was 16 degrees and the SNR improvement was 3.4dB with 10 kHz A-line rate. Microstructure of human dermis in speckle-reduced OCT images could be visualized clearer. Both systems required a rotational mirror to select the different angles backscattered from the sample and collected by the lens. Similar results were achieved by Hughes et al. [74] in which the speckle contrast ratio decreased by a factor of 2 with negligible loss of resolution. Angle selection was achieved by attaching the galvo scanner on a transversal translation stage. Nevertheless, angle variation by sequential selection [73, 74] using a rotational/translational mirror can achieve large numbers of speckle patterns but are unsuitable for endoscopic application.

Angular selection can also be parallelized, such as by array detector [72, 75, 76], joint aperture [77], Doppler-shift encoding [78] and path-length encoding [79].

Angular compounding using an array detector was first demonstrated by J. M. Schmitt [76]. Each detector received backscattered light from the sample media at a different angle and the detected signals were added incoherently (compounding). Backscattered waves in different angles were uncorrelated to each other since they experienced random optical delay. The SNR was doubled with four detectors, without significant loss in resolution.

Thomas Klein et al. developed a high-speed joint-aperture OCT [77] at MHz and demonstrated the speckle reduction capability by *in vivo* retinal imaging. The detection was split into four channels in a single acquisition. Without increasing illumination power, the collection efficiency and effective sensitivity were increased. The measured SNR increased by a factor of 1.4, which was lower than the predicted value, since the power level in the passive channels was lower than that in the active channel.

B-scan Doppler-shift encoding was proposed by Wang et al. [78]. The SNR was improved by a factor of 1.84. Quail embryo and porcine esophagus imaging experiments were conducted by this group to demonstrate an enhanced contrast of fine structures and layers after speckle reduction.

Iftimia et al. described a path length encoded angular compounding method in which each image was obtained at different incident angle encoded by path length [79]. The path length difference was introduced by putting an optical glass in

the sample arm to divide the sample beam into two beamlets. The two beamlets travelled different path length delay and illuminated the sample at different angle. Speckle pattern was suppressed through averaging these images. *In vivo* human skin images were obtained to demonstrate an increase in SNR by a factor of 1.63.

The first three approaches suffer from some loss of lateral resolution due to the split of angular aperture, while path-length encoding has decreased imaging range.

An inexpensive angular compounding technique for fiber-optic μ OCT imaging systems has not yet been reported to the best of our knowledge. This chapter presents an angular compounding technique based on fiber-optics for speckle reduction to obtain better image contrast for high resolution OCT system.

5.2 System setup

The MFOCT system is depicted in Figure 5.2. Near-infrared (NIR) illumination is provided by a broadband supercontinuum light source (Supercontinuum Source SC-OEM, YSL, Wuhan Yangtze Soton Laser Co.,Ltd) over a spectrum from 400 nm to 2400 nm with total output power > 8 W. All the optical emission > 1000 nm was blocked by a short pass filter (DMSP1000, Thorlabs). We split the source output using a 70:30 (R:T) non-polarizing cubic beam splitter (BS1), followed by a 50:50 fiber coupler (Thorlabs, TW850R5A2) to further split into the second and third channels, and directed each beam to the input of three Michelson interferometer channels. The power distribution was 30%, 35% and 35% for channel 1, 2 and 3 respectively. In each channel, we used an additional 50:50 fiber coupler to split the reference arm and sample arm. The sample arm fibers of all three channels are terminated with a v-groove array (VGA1) (VGA-8-127-0-X-14.4-4.0-2.03-S-780-5/125-3A-1-2-0.5-NF780HP, Wavelength Opto-Electronic) with 127 μ m spacing. The reference fiber length was tailored to match that of the corresponding sample fiber and the residual material dispersion was further compensated by use of fused silica blocks. Three paddle polarization controllers were used on the reference arm to adjust polarization and maximize interference fringe visibility.

As shown in Figure 5.2 (b), the end face of VGA1 were angle-polished with respect to the horizontal axis (6390.1D, ULTRAPOL) at -8, 0 and 8 degrees for channel 1, 2, and 3 respectively. In addition, all three channels are polished at 8 degrees

FIGURE 5.2: Multifiber angular compounding optical coherence tomography system schematic. SCL: Supercontinuum lightsource. L1-11: lenses. BS1: non-polarizing cube beam splitter. SMF1-3: single mode fibers. M1-3: reference mirror. ID1-3: Iris Diaphragms. G: grating. GS: galvo scanners. P1-3: polarization controllers. FC1-3: 50:50 fiber couplers, FS1-3: fused silica dispersion compensators. a) Overview system schematic. b) Probe schematic to demonstrate angle polish and angular diversity and image of angle-polished fiber array tip. Three channels were implemented. The middle channel is flat polished while the immediate adjacent channels were polished at 8 degrees. Incident angle on sample was around 3.7 degrees for the side channels at $320\ \mu\text{m}$ spacing (figure not to scale). The channel spacing is $127\ \mu\text{m}$. Blue arrows: active channels utilized in the proposed setup. c) Spectrometer multichannel alignment illustration.

with respect to the vertical axis (Figure 5.2 (b)) to remove the Fresnel reflection at the fiber tip from the spectrometer input. In the benchtop probe, all three beams were collimated by an achromat (L8, AC050-010-B, Thorlabs Inc.), redirected by a galvo scanner and focused by objective lenses (AC050-016-B, Thorlabs Inc.) into focal spots with a theoretical full-width at half maximum diameter of $3.4\ \mu\text{m}$ with slight lateral displacements of $320\ \mu\text{m}$. Interference signals obtained from each channel were directed into another v-groove array (VGA2) with channel spacing of $250\ \mu\text{m}$ (VGA-8-250-0-X-14.4-4.0-2.03-S-780-5/125-3A-1-2-0.5-NF780HP, Wavelength Opto-Electronic) after passing the 2X2 fiber coupler and collimated by an achromat (L9, AC127-025-B-ML, Thorlabs Inc.) before entering the spectrometer (Figure 5.2 (a)). The three-channel-spectrometer consisted of a 1765 lines/mm grating (PING-Sample-020, Ibsen photonics), a camera lens (Canon, EF 85mm/1.2

II) and a three-line camera (EV71YM3VCL4010-BAO, ELiiXA 3V). The signal from each channel was detected separately by each individual CMOS linear array. VGA2 was rotated by 1.35 degrees from horizontal to generate a 20 μm vertical offset to match the separation of the CMOS arrays [80]. We used ~ 3600 pixels to detect the signals with a bandwidth of 165 nm. Spectra were digitized at 12-bit resolution and transferred to the computer through camera link cables and an image acquisition board (KBN-PCE-CL4-F, Bitflow). Both camera and the galvo scanner were synchronized by a computer-generated trigger signal at an A-line rate of 5 kHz.

5.3 System characterization

Standard OCT image processing was independently performed for each channel (background subtraction, interpolation and mapping from wavelength- to k- space and Fourier Transform [81]). To compensate for the fixed lateral displacement between foci, image registration was performed as a one-time calibration. The lateral shift was calibrated by scanning and matching a standard USAF-1951 resolution target and axial shift was optically compensated by adjusting the reference mirror position. Finally, images of different angles were compounded linearly and displayed on a logarithmic scale.

The theoretical axial resolution at 860 nm center wavelength of 165nm bandwidth would be 2 μm for a perfectly Gaussian spectrum, though the actual axial resolution was slightly degraded because of the combined effects of the non-Gaussian source spectrum and the camera response. The experimental axial resolutions were measured to be 2.6 μm , 2.9 μm , and 3.2 μm respectively for the three channels (Figure 5.3 (b)). The difference in axial resolution may come from spectrometer misalignment and spectrum calibration difference. As group 7 element 2 of USAF resolution target can be resolved for all channels, the lateral resolutions were measured to be approximately 3.50 μm .

To characterize the sensitivity of the system, we measured the signal-to-noise ratio (SNR) using a partially-reflecting mirror sample (-14 dB reflectivity) attenuated by a 14 dB neutral density filter. The path length difference between the sample and reference mirrors was maintained at 50 μm . The average incident power on

FIGURE 5.3: a) Multichannel spectrometer reference spectrum for the three channels respectively, obtained from the spectrometer configuration. Camera response introduces fringes in the spectrum. b) Point spread functions for each individual channel. $\sim 3\mu\text{m}$ axial resolution in air was achieved

the sample was approximately 5 mW from each channel at $180\ \mu\text{s}$ exposure. The sensitivity for each camera line sensor was measured to be 98.5 dB, 100.1 dB, and 99.8 dB respectively.

5.4 Imaging experiment and discussion

Ex vivo imaging experiments on rat esophagus were conducted at a frame rate of 10 Hz with 512 lines per frame per channel. Wild-type rat esophagus fresh tissue was dissected, kept in PBS solution and imaged within one hour of sacrifice. OCT images were acquired with the image size of 315×315 (axial $\times$ transverse) pixels, corresponding to $600\ \mu\text{m} \times 430\ \mu\text{m}$.

Figure 5.4 compares images obtained without (left) and with (right) angular compounding technique. Spatial compounding was also implemented with varying frame numbers, $N=1, 3, 30$ and 100 , and its speckle reduction effect were compared with that of angular compounding. Qualitatively, the amount of speckle reduction imparted by spatial compounding was minimal for $N < 10$; speckle remained largely correlated within this window. At $N=30$, the contrast between layers improved, and a speckle reduction effect was more evident. Notably, a comparable speckle reduction result was achieved in a single frame by compounding images obtained by the three individual channels. By averaging different frames of angular compounded images, better image contrast and SNR were observed even with a single frame, since three uncorrelated speckle fields are available in a single shot.

FIGURE 5.4: Comparison of speckle reduction effect using spatial compounding for single channel and multichannel respectively. Better image contrast was observed for multichannel system. a)-d): Left column: spatial compounding for single channel. Right column: spatial compounding for multichannel across 1, 3, 30, and 100 frames respectively. Ep: Epithelium. LP: Lamina Propria MM: Muscularis mucosae.

FIGURE 5.5: Cross-sections of rat esophagus with 3-frame averaging applied. a) Single-channel result is highly obscured by speckle. b) Multichannel image exhibits reduced speckle and better delineation (yellow arrows) that are concealed in (a). c) Representative histology.

FIGURE 5.6: Regions of interest for SNR, CNR and ENL analysis

One of the major drawbacks of spatial compounding speckle reduction is the degradation in lateral resolution. Averaging across more than 30 adjacent frames degrades resolution. With the multifiber angular compounding probe design, features were revealed that were originally obscured by speckle. Figure 5.5 compares images obtained from a single channel (left) and multichannel (right), both averaged across 3 adjacent frames. The layered structure of rat esophagus was more distinguishable in speckle-reduced image (Figure 5.5 (b)). The epithelium was better delineated from the overlying mucus and lamina propria beneath the basement membrane, which is not clearly distinguished with the conventional single-channel technique (Figure 5.5 (a)) due to speckle noise. This image quality improvement is confirmed by a representative histology image (Figure 5.5 (c)).

The image quality was also evaluated using well-established speckle-reduction performance metrics [82–86] for the esophagus images in Figure 5.5, including signal-to-noise (SNR), contrast-to-noise ratio (CNR) and equivalent number of looks (ENL) defined by the Equations 5.1, 5.2 and 5.3. The CNR gives the contrast

	SINGLE	COMPOUNDED	IMPROVEMENT
SNR	43.81 dB	45.32 dB	1.51 dB
CNR	3.95	6.26	1.58
ENL	183.59	463.99	2.53

TABLE 5.1: SNR, CNR, ENL comparison for with and without multifiber speckle reduction technique

between a region of interest and a region of background noise, while the ENL measures the smoothness of speckle-corrupted homogenous region.

$$SNR = 10 \times \log_{10}(\max \frac{S_{lin}^2}{\sigma_{lin}^2}) \quad (5.1)$$

$$CNR = \frac{1}{R} \sum_{r=1}^R \frac{\mu_r - \mu_b}{\sqrt{\sigma_r^2 + \sigma_b^2}} \quad (5.2)$$

$$ENL = \frac{1}{H} \sum_{h=1}^H \frac{\mu_h^2}{\sigma_h^2} \quad (5.3)$$

In the above equations, S_{lin} and σ_{lin}^2 are the magnitude of image and variance of background noise region from linear OCT images, μ_b and σ_b^2 are the mean and variance of the same background from logarithmic OCT images, μ_h and σ_h^2 are the mean and variance of all the homogeneous regions of interest (H), and μ_r and σ_r^2 are the mean and variance of all the regions of interest (R), including the homogeneous regions (H) from logarithmic OCT images also. The distance between adjacent A-lines was $1.37 \mu\text{m}$, which is less than half of the beam diameter, thus the window size was chosen to be 5×5 pixels for $H = 5$ and $R = 9$. The homogenous regions were chosen within a single tissue layer whereas the non-homogenous regions were chosen at tissue layer boundaries across two different tissue layers as shown in Figure 5.6.

The SNR, CNR and ENL for single and angle-compounded OCT images were investigated. The center channel was selected for single channel evaluation because its sensitivity and lateral resolution were the highest among the three channels. SNR, CNR and ENL were evaluated and averaged over 50 frames (Table 5.1).

The SNR improved by 1.51 dB for angle-compounded image. We attribute the difference between the experimental values to the theoretical value of 2.39 dB to the coherence of the speckle noise in the three channels. The CNR improved by 1.58 for angular-compounded image. ENL also has significant improvement by a factor of 2.53 for angular-compounded image.

5.5 Summary and outlook

In conclusion, we successfully demonstrated MFOCT system for speckle reduction in spectral domain OCT. A fiber optic multichannel system is better than a free space multichannel system for its ease of construction and maintenance, which also increases clinical usability. The key element – angle polished fiber array – can be implemented in an endoscopic or intravascular probe by reducing the channel spacing or use of a multicore fiber. The speckle-reduced images exhibited better image contrast, SNR, CNR and ENL compared to the original images without loss of resolution. These advances in image quality will be particularly useful for visualization of cellular and subcellular structures which suffer most from speckle noise. While substantial speckle reduction was seen with our present system, improving system performance by increasing fiber density using a multicore fiber will be a subject of future development. In addition, we used a relatively low imaging speed in this proof-of-concept study, which is not enough for most of *in vivo* applications. This data throughput issue can be solved by improving imaging acquisition and display program.

Chapter 6

Conclusion

6.1 Summary of work

This dissertation presents four major contributions.

The first contribution is development of a method to monitor cilia regeneration and ciliary function restoration in adult mice using μ OCT imaging technique. We successfully monitored the cilia regeneration based on the epithelial thickness, cilia coverage, length, beat frequency, coordination and generated flow with the help of μ OCT imaging at 80 frames per second (fps) for up to 52 days. μ OCT imaging enabled us to categorize the procedures of cilia recovery into two phases: regeneration and refinement. The regeneration phase is for the development of ciliated cells and growth of cilia to adequate coverage and length to support MCT. The refinement phase is for the cilia to improve alignment and beat coordination. We uncovered that the average cilia-generated flow increased significantly at refinement phase but there was little change in epithelial thickness, cilia coverage, length, and beat frequency during this process. We also found the speed increase coincided with the cilia beat coordination enhancement. This finding was confirmed by an *in vitro* airway recovery experiment. An overshoot in CBF was observed at the late regeneration phase or early refinement phase. It could be a sign of lag in cilia coordination at the early stage of regeneration.

The most important contribution of this dissertation is the design, prototyping and testing of the μ OCT flexible endobronchial probe for clinical translational studies

to image MCT in airways of adult patients for the purpose of understanding the MCT phenotypes of airway diseases such as cystic fibrosis. The μ OCT probe was composed of three main segments: optics, sheath and ergonomics. Firstly, in the optics segment, the μ OCT probe integrated both the reference arm and the sample arm into a common-path configuration to minimize the material dispersion and ensure optimal optical quality. Secondly, the dimensions of the outer sheath were 1.75 mm inner diameter (ID) and 2.4 mm outer diameter (OD). The new design allows the probe to be easily fitted into the instrument channel of a standard bronchoscope. Lastly, a customized ergonomic attachment was designed which introduces the probe into the instrument channel of the bronchoscope and fixes its relative position to top of the bronchoscope. We performed *ex vivo* μ OCT imaging of mouse trachea using both the benchtop μ OCT instrument as well as the flexible probe attached to a typical SD-OCT platform. In both cases, microanatomical features such as cilia on the apical surface were clearly distinguishable, which indicates that the flexible bronchial probe has comparable imaging performance to that of the benchtop system. We also validated the μ OCT system, which included the console and the airway probe, was safe to conduct experiment *in vivo* without causing laser exposure damage prior to use in human patients. The first *in vivo* swine experiment with this flexible endobronchial probe showed promising results in terms of optical performance. We successfully developed a miniaturized flexible μ OCT probe suitable for imaging the epithelial functional microanatomy *in vivo*. Translation to human subjects will greatly benefit the study of CF pathogenesis and the assessment of the efficacy of experimental therapeutics. This flexible endobronchial μ OCT probe was the first step towards clinical μ OCT application and opens possibility to image cilia and mucociliary transport in small distal airways *in vivo*, where the pathogenesis of lung diseases such as CF and chronic obstructive pulmonary disease are believed to originate, allowing the underlying mechanisms of these pathologies to be studied. Further, patient-specific imaging of mucociliary function would allow personalized monitoring and tailoring of interventional treatments, which would be particularly valuable in diseases such as CF that frequently require cocktail pharmacologic therapies.

The third contribution is using a dual-spectrometer configuration to improve the axial resolution of μ OCT. Spectra from the two spectrometers were combined to achieve an axial resolution of 1.27 μ m in air. We have validated the axial resolving power of the proposed system in zebra fish larvae by visualizing cellular structures

FIGURE 6.1: Driveshaft with different bend radius during scanning: (a) when scanner pull, the driveshaft is under tension (b) when scanner push, the driveshaft is under compression

in vivo. The dual spectrometer design makes it possible to improve axial resolution using NIR radiation and gain improved penetration by use of longer center wavelength.

The last contribution of this dissertation is a MFOCT system for speckle reduction in spectral domain OCT. The speckle-reduced images exhibited better image contrast, SNR, CNR and ENL compared to the original images without loss of resolution. A fiber optic multichannel system is better than a free space multichannel system for its ease of construction and maintenance, which also increases clinical usability. The key element – angle polished fiber array – can be implemented in an endoscopic or intravascular probe by reducing the channel spacing or use of a multicore fiber.

6.2 Outlook for future studies

6.2.1 μ OCT flexible endoscopic bronchial probe

In the previous studies, we have verified the imaging capability of the flexible μ OCT endoscopic probe *in vivo* in swine model. There are some limitations of current design and we recommend the following steps for performance improvement.

6.2.1.1 Scan range

The scan range reduced with respect to bending radius due to backlash originated from the space between the outer surface of the driveshaft and the inner surface of the sheath and the fact that the scanner was located at the proximal end of

FIGURE 6.2: Picture of bending test setup

Bend Ratio	1.00	0.96	0.93	0.89	0.85	0.82	0.78	0.75	0.71	0.67
Scan Range (μm)	840	800	720	850	510	360	250	210	160	0

TABLE 6.1: Table of ending test result: scan range (μm) versus bending ratio FIGURE 6.3: Bending test result: bending ratio versus scan range (μm)

the probe. When the scanner retracted and pulled the driveshaft, the driveshaft was under tension and pressed against the lower inner wall as shown in Figure 6.1 (a). When the scanner extended and pushed the driveshaft, the driveshaft was under compression and pressed against the upper inner wall as shown in Figure 6.1 (b). Therefore we characterized the reduction in the scan range with respect to different bending radius and found the scan range was reduced significantly as bending radius decreased. The test setup was shown in Figure 6.2. The bending ratio was measured as the straight-line distance between the bronchoscope distal tip and proximal end over the actual length of it.

FIGURE 6.4: Schematic of new probe tip design integrating microspring at distal end

In typical clinical experiments, the bending at the neck caused the scan range to reduce by half, from $400\mu\text{m}$ to around $200\mu\text{m}$ (Figure 6.3 and Table 6.1).

To solve this issue, a distal scan mechanism could be employed. However miniaturized scanner of diameter less than 2.4 mm is not available yet and it may introduce electrical safety issues. Therefore, we propose to have a microspring attached at the distal end of the probe connecting driveshaft and sheath (Figure 6.4). The spring should always be loaded under tension, so as the driveshaft. The result is that instead of having the scanner push and pull the driveshaft during scanning, it pulls the driveshaft at difference forces and the driveshaft is in the position as in Figure 6.1(a) at all times.

Further experiment should be conducted to validate the feasibility of this design.

6.2.1.2 Imaging window

Exact scan range information was not available in previous experiments. We propose the following method for improvement:

1. Laser marking

Current imaging window was made of heat shrink tube. Thin lines of known spacing could be marked onto it using high power laser. Precise control of the laser power, exposure time and line spacing will be the most challenging part for this method.

2. Heat shrink tube printer

Similar to solution 1, we could also use heat shrink tube printer to mark the imaging window. We have explored this method and found current commercial heat shrink tube printer substrates were limited on specific materials, sizes and their inks were classified as slight hazardous (class 1) for Lung and hazardous (class 2) for cancerous. However, this method may become available with the heat shrink tube printer technology development.

3. Speckle decorrelation

There was speckle observed inside the imaging window. Decorrelation of the speckle between adjacent A-lines may provide information of the actual spacing between each adjacent A-line. This method requires further exploration

6.2.1.3 Driveshaft and sheath

During the *in vivo* swine experiments, images were occasionally lost due to the misalignment between the optical path and the imaging window, so the sample beam was blocked by the sheath. This was caused by the torque along the driveshaft.

We propose to customize both driveshaft and sheath into D-shaped as shown in Figure 6.5 to ensure perfect alignment between the optical path and the imaging window.

6.2.1.4 Motion artifacts

Motion artifacts were the major issues discovered during *in vivo* experiments, which were introduced by two main factors: heartbeat and breath. As discussed in Section 3.8, better mechanism to fix the probe relative position to the tissue without direct contact to disturb airway physiology functions is desired.

6.2.2 Dual spectrometer system

The dual-spectrometer design could be easily integrated into any SDOCT systems, including but not limited to the clinical μ OCT system with flexible μ OCT endoscopic probe and MFOCT system described in this dissertation, for resolution enhancement.

FIGURE 6.5: Illustration of the cross-sectional image of D-shaped probe drive-shaft and sheath

6.2.3 MFOCT System

In the MFOCT system, the multichannel probe could be further miniaturized for endoscopic applications by integrating multicore fiber. The proposed system would be the first speckle reduction endoscopic OCT probe in the world to the best of the author's knowledge.

Firstly, the $127\ \mu\text{m}$ fiber core spacing caused the three beams to propagate with such a diverging angle after passing the probe collimation lens, that an objective lens with a large aperture was required to enclose all three input beams. Consequently, this optical prearrangement rules out the possibility of achieving high lateral resolution using low cost objective lens. Secondly, the speckle reduction effect scales with the number of channels, which was only three in the current setup. Both problems could be addressed by replacing the fiber array with more compact multibeam endoscopic probe configuration similar to [87] or a multicore fiber. These approaches would increase fiber density, increasing the number of channels and reducing space between different channels as well as enabling a more compact

FIGURE 6.6: Schematic of angular compounding μ OCT system with multicore fiber probe

and flexible imaging probe. Lastly, we used a relatively low imaging speed in this proof-of-concept study, which is not enough for most of *in vivo* applications. This data throughput issue can be solved by improving imaging acquisition and display program. Increased number of channels, reduced space between different channels and improved data acquisition speed in this design would improve the technical feasibility of implementation of the technique and contribute significantly to *in vivo* clinical translational studies.

We propose to use the following configuration (Figure 6.6) to integrate the novel multicore fiber into the angular compounding μ OCT system as endoscopic imaging catheter. Similar to the current design, the distal end of the multicore fiber will be polished to have certain angles as indicated by the red arrow in figure 6.6 to collect backscattered signals from the same sample volume at different angles. The multicore fiber has seven cores and the distal end of it can be polished into spherical shape to utilize all seven cores. Here we used three of the channels for illustration. Light coming out of the source will be collimated into parallel beams by the collimation lens L1. Then it will be split into three channels by 3 beam splitters BS1-3. L2-4 are used to couple the light to the single mode fibers.

Two designs of the catheter are proposed to realize 360 degree scanning of biological tissues. In the case of option 1, a fiber rotary joint is attached in between of the single mode fibers and FA1 (fiber array 1) to rotate the catheter. The three beams coming out of the fiber array will be coupled into respective cores of the multicore fiber by a pair of lens. The distal end of the multicore fiber is polished so that lights coming out of it propagate at different angles. Then lights will be focused by the GRIN lens. A micro non-polarizing cube beam splitter (BS4) is used to separate the light into reference beam and sample beam. A reference mirror M3 is placed at the focus plane of the reference arm whereas sample to be imaged is placed at the focus plane of the sample arm. Light backreflected by M3 and backscattered by the sample is recombined by BS4 and interference signals propagate back along the same paths to BS1-3. Then they are collected by fiber array 2 (FA2) and dispersed by a grating before detected by a three line CCD camera. Because the rotation driving force comes from the fiber rotary joint for option 1, every component attached after the fiber rotary joint will rotate together during 360o scanning.

The only difference between option 1 and option 2 is the rotational mechanism. The driving force of rotation of option 2 is a micromotor attached at the distal end of the fiber probe and only micro cube and M3 will rotate during 360o scanning. The driving forces of both catheter options are presented by the pink shaded area.

Author's Publications

Journal Articles

- **Dongyao Cui**, Kengyeh Chu, Biwei Yin, Timothy N. Ford, Chulho Hyun, Hui Min Leung, Joseph A. Gardecki, George M. Solomon, Susan E. Birket, Linbo Liu, Steven M. Rowe, Guillermo J. Tearney, “Flexible, high-resolution μ OCT endobronchial probe toward *in vivo* imaging of cilia,” *Optics Letters* 42 (4), 867-870 (2017).
- **Dongyao Cui**, En Bo, Yuemei Luo, Xinyu Liu, Xianghong Wang, Si Chen, Xiaojun Yu, Shi Chen, Ping Shum, Linbo Liu, “Multifiber Angular Compounding Optical Coherence Tomography for Speckle Reduction,” *Optics Letters* 42 (1), 125-128 870 (2017).
- **Dongyao Cui**, Xinyu Liu, Jing Zhang, Xiaojun Yu, Sun Ding, Yuemei Luo, Jun Gu, Ping Shum, and Linbo Liu, “Dual spectrometer system with spectral compounding for 1- μ m optical coherence tomography in vivo,” *Optics Letters* 39, 6727-6730 (2014).

Conference Proceedings

- **Dongyao Cui**, En Bo, Yuemei Luo, Xinyu Liu, Xianghong Wang, Si Chen, Xiaojun Yu, Shi Chen, Ping Shum, Linbo Liu, “Multichannel angular compounding SDOCT for speckle reduction” *Photonics Global Conference (PGC)*, Singapore, Aug, 2017.
- **Dongyao Cui**, En Bo, Yuemei Luo, Xinyu Liu, Xianghong Wang, Si Chen, Xiaojun Yu, Shi Chen, Ping Shum, Linbo Liu, “Optical coherence tomography multichannel probe design for speckle reduction,” *International Conference on Biophotonics (ICOB)*, Perth, WA, Australia, Apr, 2017.

- **Dongyao Cui**, Kengyeh K. Chu, Timothy N. Ford, Chulho Hyun, Hui Min Leung, Biwei Yin, Susan E. Birket, George M. Solomon, Steven M. Rowe, Guillermo J. Tearney, “Imaging demonstration of a flexible Micro-OCT endobronchial probe,” *Photonics West BiOS, SPIE*, San Francisco, CA, USA, Feb, 2017.
- **Dongyao Cui**, Hui Min Leung, Kengyeh K. Chu, Timothy N. Ford, Chulho Hyun, Biwei Yin, Kanwarpal Singh, Susan E. Birket, George M. Solomon, Steven M. Rowe, Guillermo J. Tearney, “Demonstration of mouse trachea imaging with a flexible Micro-OCT (μ OCT) endobronchial probe,” *North American Cystic Fibrosis Conference (NACFC)*, Orlando, FL, USA, Oct, 2016.
- **Dongyao Cui**, Kengyeh K. Chu, Carolin I. Unglert, Timothy N. Ford, Robert W. Carruth, Daryl Hyun, Kanwarpal Singh, Susan E. Birket, George M. Solomon, Steven M. Rowe, Guillermo J. Tearney, ‘Flexible micro-OCT endobronchial probe for imaging of mucociliary transport,’ *Photonics West BiOS, SPIE*, San Francisco, CA, USA, Feb, 2016.
- **Dongyao Cui**, Liu, Xinyu, Zhang, Jing, Yu, Xiaojun, Sun, Ding, Luo, Yue-mei, Gu, Jun, Shum, Ping, Liu, Linbo, ‘One-micron resolution optical coherence tomography (OCT) *in vivo* for cellular level imaging,” *Photonics West BiOS, SPIE*, San Francisco, CA, USA, Feb, 2015.
- **Dongyao Cui**, Xinyu Liu, Xiaojun Yu, Ping Shum, Linbo Liu, ‘Micro-optical coherence tomography (μ OCT) *in vivo*,” *Optics & Photonics Congresses (Imaging and Applied Optics)*, OSA, Seattle, WA, USA, Jul, 2013.

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